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ANNUAL A.D.S.A. MEETING

North Carolina State College, Raleigh

June 17–19, 1958

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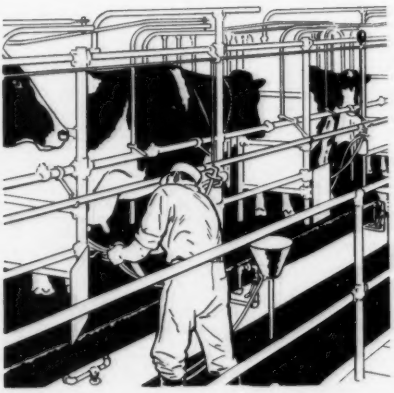

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PEOPLE AND EVENTS

Georgia News

The Southern Fluid Milk and Ice Cream Cost Analysis Clinic will be held May 5-7 in the Univ. of Georgia Center for Continuing Education.

Purpose of the clinic is to bring together key personnel in this field, so that they may study in detail ingredients, personnel, quality control, merchandising, and sales costs. The program also will include sessions on taxes, depreciation schedules, and Social Security problems.

The two-day event is sponsored by the Dairy Dept. of the University, in cooperation with the Georgia Center for Continuing Education. More information may be obtained from H. B. HENDERSON, Dairy Dept., Univ. of Georgia, Athens.

At the meeting of the Georgia Chapter of the International Association of Milk and Food Sanitarians held October 30-31 at the University, W. WEAVER of Columbus was honored as the outstanding milk sanitarian of the year and E. GEORGE of Perry, Georgia, as the outstanding food sanitarian.

The Graduate Faculty of the Univ. of Georgia has approved the offering of instruction leading to the Doctor of Philosophy degree in three fields, including parts of dairying. They are Animal Science with a major in animal nutrition, Bacteriology, and Food Technology and Dairy Manufactures.

This institution will be one of a very few in the South which confers the Ph.D. degree in bacteriology. A recent consultant in biological sciences referred to the equipment in bacteriology as "fabulous." The staff consists of ten members, including microbiologists in the Bacteriology, Dairy, Food Technology, Agronomy, and Veterinary Hygiene Depts.

For Animal Science, there are 12 men holding the Ph.D. degree with major emphasis in animal nutrition, and several others in related areas, available for instruction. The facilities and personnel of the Animal Husbandry, Dairy, and Poultry Depts. may be used for instruction and research.

No other institution in the South offers the Ph.D. degree in Food Technology, and only one other offers the degree in the field of Dairy Manufactures. A staff of five men holding the Ph.D. degree and four with the M.S. degree, and the combined facilities of the Dairy and Food Technology Depts., are available in this program.

The University Library recently occupied one of the most modern buildings of its kind and has greatly enlarged its reference material. The Chemistry and Animal Science Buildings in the new Science Center are already under construction and the immediate erection of the Biological and Food Technology units has been approved by the Board of Regents. The Dairy Science Building erected in 1940 has splendid laboratory facilities in dairy manufactures.

The University Dairy has just put into operation a combine milking machine which delivers the milk under vacuum to a 500-gal. refrigerated tank. The creamery has purchased a 500-gal. capacity tank truck to pick up the milk from the dairy and from other sources of supply. Other additions to the creamery equipment are an evaporative condenser for the refrigeration system, and an 800-gal. milk-storage tank.

Fairmont Promotes Hartley

C. B. HARTLEY has been named quality control manager and assistant to the production vice-president for Fairmont Foods, Omaha, Neb. He took over his new position Jan. 1, 1958.

Before joining Fairmont Foods, Dr. Hartley was with the Kraft Foods Company. He received his B.S. degree in dairy industry and his Ph.D. degree in dairy bacteriology from the Univ. of Minnesota, St. Paul.

Announce Training Program for Steroid Biochemistry

Applications are now being accepted for the third course in the training program for steroid biochemistry which will begin October 1, 1958. The training program is sponsored by the National Cancer Institute of the National Institutes of Health to provide specialized training for individuals interested in steroid investigations.

The program is conducted by personnel at the Worcester Foundation for Experimental Biology with the Dept. of Chemistry, Clark Univ., Worcester, Mass., and the Dept. of Biochemistry, College of Medicine, Univ. of Utah, Salt Lake City.

Two groups of candidates will be selected for training. Postdoctoral candidates having an M.D. or a Ph.D. degree will receive $5,000 for a one-year training period extending from Oct. 1, 1958, through Sept. 31, 1959. The course will consist of laboratory instruction and lectures covering the theoretical and practical aspects of steroid research, and a period of research on a steroid problem with an established investigator.

Candidates having a B.S. or an M.S. degree, or equivalent training, will receive $1,500 for a six-months' period extending from Oct. 1, 1958, through March 31, 1959. The predoctoral program is intended to provide competency in

the analysis of steroid substances for research and clinical laboratories.

The closing date for applications will be June 1, 1958. Requests for applications should be made to the Training Program for Steroid Biochemistry, Dept. of Chemistry, Clark Univ., Worcester, Mass., or to Dr. KRISTEN EIK-NES, Dept. of Biochemistry, College of Medicine, Univ. of Utah, Salt Lake City.

Wisconsin News

Shell fragments on the Anzio beachhead which almost killed ROBERT T. HILL, Seymour, Wis., could not wreck his hopes of becoming a farmer.

But it was a frustrating seven years between his discharge from an army hospital in March, 1945, and the start in July, 1952, of farm-training under the U. S. Vocational Rehabilitation Act for seriously disabled World War II veterans, administered by the Veterans Administration.

When the former machine gunner was wounded, it caused a functional injury to his central nervous system. After months of hospitalization, he regained his physical strength but suffered from occasional black-outs, which made it unsafe for him to operate machinery and seemed to rule out farming.

However, he couldn't stay away from the soil. He worked as a farm hand at non-dangerous chores while continuing to receive VA medical treatment.

Finally, seven years after his army medical discharge, he did two things: He bought a farm and sought farm training combined with classroom instruction under the U.S. act.

After a series of counseling interviews, aptitude and medical tests, VA counselors and medical consultants agreed Robert could train as a farmer.

His 48-month training program was planned to avoid operation of dangerous machinery. He was fortunate in having two brothers operating nearby farms, which permitted an exchange of work. They did the hazardous tasks on Robert's farm.

By the time he completed training in July, 1956, he had developed an outstanding dairy herd, which produces an average of over 400 lb. of butterfat per cow.

Hill was wounded January 30, 1944, but he says he's going to observe the anniversary in 1958 for another reason: "I'll be making the last mortgage payment on the farm about that time."

A 51-ft. tall cylinder of stainless steel was raised into place at the west end of Babcock Hall at the Univ. of Wisconsin, Madison, last summer, signalling the beginning of an extensive research project to improve some of Wisconsin's major dairy products.

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Now enclosed with brick, and opening into the dairy product processing rooms in Babcock Hall, the 8-ft. wide cylinder is an experimental dryer for dairy and other food products—the first of its kind at any university. As it was being put into place, the tower had all the outward characteristics of a guided missile ready for launching.

Development of a high-quality whole milk powder and an engineering study which aims at improving future dryer designs are the main objectives of research with the dryer.

The work is being directed by A. M. SWANSON, professor of dairy industry, and W. R. MARSHALL, JR., professor of chemical engineering. They designed the experimental unit and will be in charge of its operation.

Ohio State News

W. J. HARPER, Dept. of Dairy Technology, Ohio State Univ., Columbus, delivered a paper on "Comparative Evaluation of Hand- and Spray-Cleaned Storage Tanks" before the U. S. Public Health District Seminar. The Seminar was held Feb. 19, at the Robert A. Taft Sanitary Engineering Center, Cincinnati, O.

A. L. SHOUGH, Kroger Company, Cincinnati, has been elected president of the Ohio Dairy Products Association for 1958. Other officers elected include R. WEBNER, Cottage Creamery Company, Orrville, vice-president, and J. BURNS, Blue Valley Creamery, Columbus, treasurer.

New members of the board of trustees of the Association include: *Ohio milk distributors*—L. R. STAUFFER, Warren Sanitary Milk Co., Warren, president; E. MOSER, Med-O-Pure Dairy, Washington Court House, vice-president, and H. SEWELL, Chestnut Ridge Dairy, Akron, past-president; *Ohio butter manufacturers*—H. SPECHT, Merchants Creamery Co., Cincinnati, president; H. HAMILTON, Eldorado Creamery, Eldorado, vice-president, and R. WEBNER, Cottage Creamery Co., Orrville, past-president; *Ohio milk products manufacturers*—S. L. JOHNSONBAUGH, Defiance Milk Products, Defiance, president; R. BUEHLER, M. & R. Dietetic Laboratories, Columbus, vice-president, and A. J. GOOD, Pickerington Creamery, Pickerington, past-president; *Ohio ice cream manufacturers*—J. E. WEISKIRCHER, Ohio Valley Dairy, New Concord, president; D. A. DAVIS, The Borden Co., Columbus, vice-president, and W. BICHSEL, Goshen Dairy Co., New Philadelphia, past-president.

M. L. PEEPLES, former dairy instructor at Texas Technological College, Lubbock, is now on the staff of the Dairy Technology Dept. at Ohio State.

Mr. Peeples, a 1949 graduate from Texas Tech, will teach dairy engineering courses and work toward a Ph.D. in that field at O.S.U. He

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holds an M.S. in agriculture from Texas Tech and had worked for The Borden Co., Dallas, before joining the staff at Texas.

Massachusetts News

The Univ. of Massachusetts Dairy Farmers' Seminar was held Jan. 22-23 on the University campus at Amherst. More than 600 persons attended the two-day event.

Main speakers and their topics were N. MYRICK, editor of the *American Milk Review;* G. W. SALISBURY, head of the Dept. of Dairy Science, Univ. of Illinois, Urbana; H. WILCOX, Wilcox Sales Organization, Bergen, N. Y., and L. MCKEE, president of the American Dairy Association, Chicago.

The Annual Ice Cream Forum was held Jan. 30-31 on the University campus, with more than 130 persons in attendance. This was the largest attendance for the event in its history. The program included talks on ice cream production, promotion, sales, and costs.

An Ice Cream Short Course was held Jan. 20-29 on the University campus, with some 18 persons enrolled. Students in the course represented manufacturers throughout New England and Eastern Canada.

Recipients of awards at the short course included N. V. WHITE, Ipswich, Mass., who received an award for being the best ice cream judge; A. E. EVANS, Sayles Dairy Bar, North Attleboro, Mass., who had the best knowledge of ice cream defects, and H. E. JEWETT, Abbott's Dairy, Conway, N.H., and W. A. RIEDEL, Sanitary Farms Dairy, Houston, Tex., who were the top two students in the class.

The course was under the direction of F. E. POTTER and other staff members of the Univ. of Massachusetts Dairy Dept.

Illinois News

C. DUPUY, a 1948 graduate of the Univ. of Illinois, Urbana, has accepted a position with Foremost Dairies, Philadelphia, Pa. He was formerly with the Midwest Dairy Products Co., Memphis, Tenn.

G. K. MURTHY has joined the milk division of the Robert A. Taft Sanitary Engineering Center, Cincinnati, O. Dr. Murthy received his Ph.D. degree in dairy technology from the Univ. of Illinois in 1956. His wife, Lalitha, who received her Ph.D. in dairy technology from Illinois in 1957, is now with the research division of the National Distillery and Chemical Corp. in Cincinnati.

Kansas State News

The 18th Annual Meeting of the Kansas Butter and Cheese Institute was held Feb. 6, 1958, at Wichita.

Officers elected for the coming year were G. C. FREEBY, Neosho Valley Co-op, Erie, president; J. G. VESS, JR., Linwood Creamery, Wichita, vice-president, and W. H. MARTIN, Kansas State College, Manhattan, executive secretary.

Speakers at the event were S. D. FINE, chief of the Kansas City District of the U. S. Food and Drug Administration; R. FREELAND, secretary of the Kansas State Board of Agriculture, Topeka, and L. P. MCCOUN, president of the Sugar Creek Creamery Co., Danville, Ill.

E. E. BARTLEY, who received the American Feed Manufacturers' annual award in 1957, presented a paper on "The Role of Dehydrated Alfalfa in Dairy Cattle Rations" at the Annual Meeting of the American Dehydrators Association. The meeting was held Feb. 6-8 at Colorado Springs, Colo.

Clarence J. Babcock Dies

CLARENCE J. BABCOCK, a long-time national leader in milk marketing, died in Washington, D. C., on February 25. "C. J." was Director of of the Foreign Agricultural Service's Dairy and Poultry Division, USDA. He joined the Department in 1917 and had continuous government service since then, including Army service in both World Wars.

A native of Medina County, Ohio, Mr. Babcock graduated from Ohio State University, in Columbus, in 1916 with a B.S. in agriculture. His first job with the USDA was in the Bureau of Animal Industry as a scientific assistant in dairying.

An extremely prolific writer, he wrote over 100 papers on technical subjects, mainly dairy, during his long career.

During World War II, Mr. Babcock was a Lieutenant Colonel in the U. S. Army Sanitary Corps and in charge of the sanitation of milk supplies. He received the Legion of Merit award for his outstanding work.

In 1956 he was the official U. S. delegate to the 13th International Dairy Congress in Rome. One of his special interests was the International Contest in Judging Dairy Products for U. S. and foreign students. He became associated with this contest in 1926 and directed it from 1941 till his death.

Mr. Babcock was a member of the American Dairy Science Association, the International Association of Milk Sanitarians, and the Dairy Technology Society of Washington, D. C., and Maryland.

Secretary of Agriculture Ezra Taft Benson recently commended Mr. Babcock for his studies on technical and economic aspects of the U. S. dairy industry.

Food Congress to Be June 8–12

The Inter-American Food Congress will be held June 8-12 in Bal Harbour, Fla. The three-

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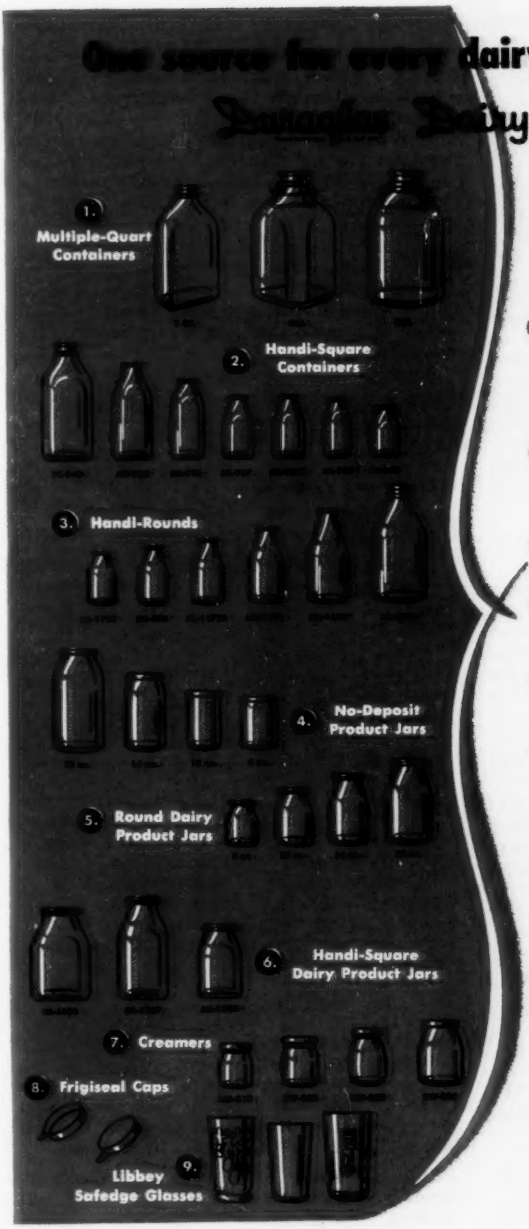

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day program will be devoted to discussions on marketing, government and industrial research, quality control, sanitation, nutrition, citrus and tropical food products, food canning, frozen foods, and poultry and meat products.

The program for the dairy section, of which A. C. FAY, North Miami, Fla., is chairman, will include the following speakers and topics: R. J. WERNER, executive director of the Milk Industry Foundation, "Program of the Milk Industry Foundation in the Merchandising of Fluid Milk in North America"; J. J. MOJONNIER, Mojonnier Bros Co., "Modern Advances in Dairy and Food Processing Equipment"; G. H. HOPSON, DeLaval Separator Co., "Pipeline Milkers and Bulk Handling of Milk on Farms"; G. TWOROGER, president of the Expert Dairy Service, "Synychronized Plant Operation"; C. B. SHOGREN, president of Klenzade Products, "Chemical Aids in Dairy and Food Plant Sanitation," and J. D. FAULKNER, chief of the Milk Section, U. S. Public Health Service, "Program of the U. S. Public Health Service in Control of Milk Supplies in the U. S."

C. W. England Convalescing

C. W. ENGLAND, director of The C. W. England Laboratories, Washington, D. C., is recovering from a serious leg and hip fracture suffered while he was on vacation in Ocean City, Md., last August. He was taken from the hospital to his home on Jan. 28, and writes that it will be early summer before he will be able to walk on the leg which was broken.

Before opening his own consulting laboratories, Dr. England was professor of Dairy Manufacturing at the Univ. of Maryland, and later director of research for the C. Y. Stephens Dairy and Poultry Industries, Washington, D. C. He has been active in the A.D.S.A. and the International Association of Milk Sanitarians.

West Virginia News

The Annual Meeting and Sale of the Ayrshire Breeders Association will be held May 5-7, 1958, at Jackson's Mill, Weston, W. Va.

The West Virginia Ayrshire Breeders Association will be host to the National Ayrshire Breeders Association. This will be the first time that a major breed club has held its annual meeting in West Virginia.

The annual meeting will be held May 6, followed by the annual banquet and the breed awards. The National Sale will be May 7, when some 40 of the breed's best animals will be offered for sale.

The 17th Annual West Virginia Univ. Short Course was held Jan. 6-9, 1958, at Jackson's Mill, Weston, W.Va. More than 88 persons attended the three-day session.

Babson Conducts Clean Milk Institutes

Some 20,000 institutional personnel at colleges and experiment stations, and milk-quality control people in the dairy industry, have been invited to attend the Institute for Clean Milk, a program to be conducted in 50 cities throughout the U. S. and Canada, beginning in March and continuing through June. The program is being conducted by Babson Bros. Co., Chicago.

The institutes will feature talks and discussions on the production of clean milk, a movie showing good practices in the production of highest quality milk, and displays of the latest models of sanitary milking equipment for the dairy farm.

Attendance at the event is by invitation only. Those interested in receiving invitations should contact Babson Bros. Co., 2843 West 19th St., Chicago 23, Ill., giving name, position, and mailing address.

E. O. Anderson Works in Pakistan

E. O. ANDERSON, Professor Emeritus, Univ. of Connecticut, Storrs, is currently working with an educational group in Lahore, Pakistan, under a cooperative arrangement with Washington State College, Pullman.

This group is working on a plan to establish a land-grant type of university in Pakistan. Both administration and the faculty are to be American.

A. C. Fay Lives in Florida

A. C. FAY, former director of laboratories for H. P. Hood and Sons Milk Co., Boston, Mass., has retired to North Miami, Fla., where he is currently revising the Milk Industry Foundation Laboratory Manual. Before joining H. P. Hood and Sons, where he was laboratory director for 21 years, he was in the Dairy Bacteriology Dept. at Kansas State College, Manhattan.

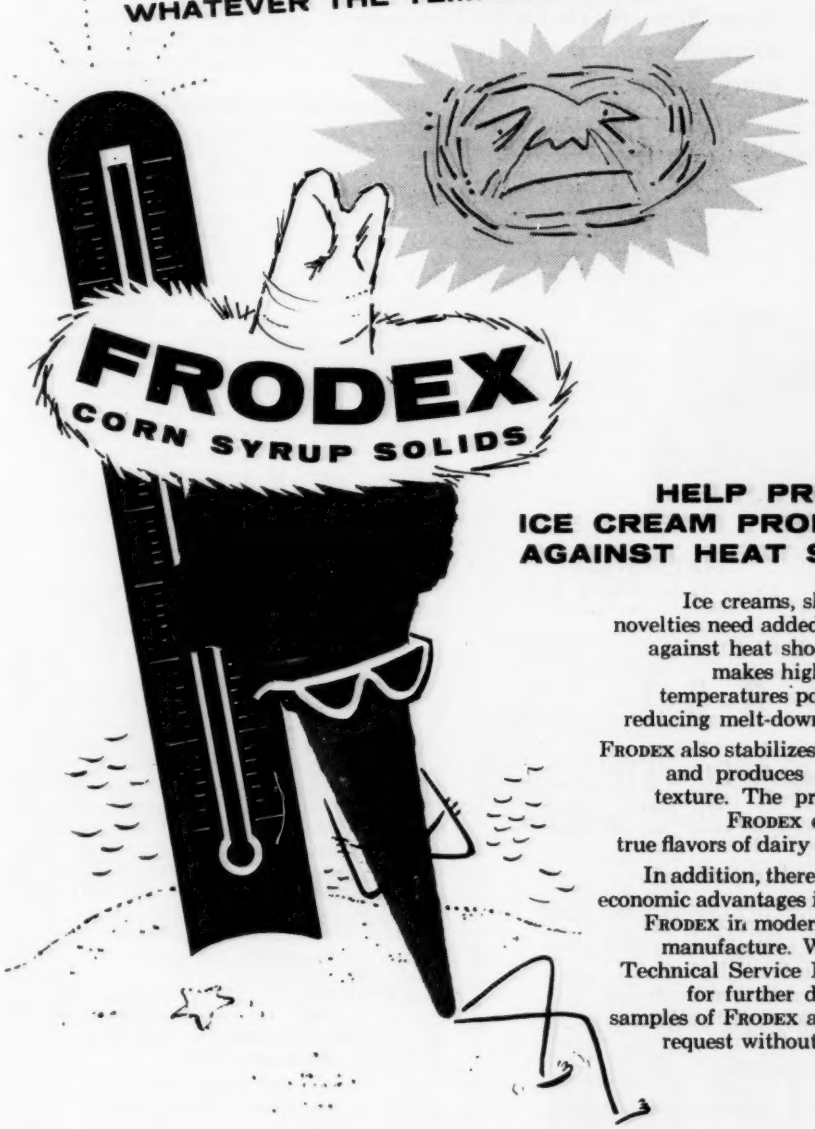
Cornell News

R. P. MARCH and J. C. WHITE of the Dairy Industry Dept., Cornell Univ., Ithaca, N. Y., have been conducting a series of training programs for bulk-tank truck drivers. More than 360 persons have participated in the 12 sessions held to date in various sections of the state.

Subjects covered in the program are properties of milk, bacteriology of milk, cleaning milk-handling equipment, procedures for drivers, and judging milk flavor and odor.

Representatives of the New York City and New York State Depts. of Health and the Dept. of Dairy Industry at Cornell met recently to plan a survey of pipeline milkers.

WHATEVER THE TEMPERATURE . . .



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***10th ed. Standard Methods—Dairy Products**

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Purpose of the survey is to study the design, installation, and cleaning procedures of the various types of farm pipeline systems used in New York, and to report on parts of systems which are not being properly cleaned mechanically.

Representatives at the meeting were E. BURTON and E. A. CRAWFORD of the New York City Dept. of Health; N. J. HOHL, New York State Dept. of Health, and W. K. JORDAN, R. P. MARCH, and J. C. WHITE, all of Cornell Univ.

R. F. HOLLAND, head of the Dept. of Dairy Industry, returned to the U. S. recently from a six-months tour in Europe under the auspices of the International Cooperation Administration of the U. S. Dept. of State.

Most of his time during the tour was spent in Greece, where he assisted the Greek Ministry of Agriculture on problems of sanitation and dairy by-products manufacture. Dr. Holland also met with agricultural officials in seven other countries of Europe.

Four faculty members are engaging in interesting projects while on sabbatical leave from the University. The following staff members are spending six months away from the campus:

W. HANSEL, Dept. of Animal Physiology, to study problems related to the metabolism of steroid hormones, using Tritium and C^{14} labeled compounds, at the Univ. of Chicago; S. J. BROWNELL, Dept. of Animal Husbandry, to assist the Greek government in implementing his recommendations for improving livestock programs, at the Ministry of Agriculture of Greece, Athens, under the auspices of the International Cooperation Administration of the U. S. Dept. of State; H. W. CARTER, Dept. of Animal Husbandry, to serve as technical adviser, at the Division of Direccion Delecheria of Argentina, Buenos Aires, under the sponsorship of the Food and Agricultural Organization of the United Nations, and J. K. LOOSLI, Dept. of Animal Nutrition, to study nutritional problems of livestock in the Florida area, at the Univ. of Florida, Gainesville.

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STUDENT CHAPTER NEWS

J. E. JOHNSTON, *Editor*

Proposed National Constitution

The proposed national constitution, as approved at the June, 1957, meeting, was circulated to the student chapters for their approval early in December. Each chapter was requested to vote on the constitution and notify your editor by February 1. As of Feb. 10, 12 of the 37 chapters had approved it, with no disapprovals received. Those chapters which have not yet voted are requested to do so as soon as possible. Two chapters raised questions about the wording of certain sections. Clarifications of this wording shall be prepared and distributed, so that final action can be taken at the annual meeting in Raleigh if the constitution is approved by the necessary majority.

Many of the chapters replying thus far have indicated that they plan to have a student delegate at the 1958 convention. This is very desirable but, where it is impossible, a faculty member of A.D.S.A. may be appointed as their representative. Thus, it should be possible for nearly every chapter to be represented at the annual meeting.

Dairy Club of Mississippi State Holds Third Annual Student-Alumni Banquet

Many of State's old grads and other guests at the Lakeside Country Club Tuesday evening, Jan. 7, saw the most successful annual Dairy Club Student-Alumni banquet given, to date.

Over 115 persons, including guests, grads, students, faculty, and their wives and dates enjoyed a delightful meal, witnessed the awarding of two scholarships, and many stayed for the dance which was the climax to a perfect program.

Willie Frank Waterer was awarded a 300-dollar scholarship by the Borden Company, presented by Mr. W. E. Schmidt, Southern field representative for the Borden Company.

The 200-dollar scholarship was awarded to James Rowzee. This was presented by Mr. Arliss Anderson, leader of extension dairying at Mississippi State.

An entertaining as well as challenging and thought-provoking speech was delivered by Mr. Hubert Garrecht, president of the Milk Industry Foundation.

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Sammie Fulgham, president of the Dairy Club, did a wonderful job as master of ceremonies.

Harold Mayo, Dale Jefcoat, Barney Wallis, Orie Lane, and Sammie Fulgham were recognized at the banquet for the tremendous amount of work and time spent to make a success of the event.

VPI Dairy Club Annual Convention

At the forty-third annual convention of the Virginia Dairy Products Association, Jan. 20-21, three V.P.I. students gave short speeches explaining views of students upon receiving scholarship grants. The students were accompanied to the convention, which was held at Hotel Roanoke in Roanoke, Virginia, by Dr. G. C. Graf, Head of the V.P.I. Dairy Science Department, and other professors in Dairy Science at V.P.I. The three students participating in the program were Sidney Spahr from Bristol, Paul Kipps from Aroda, and Don Sowder from Rocky Mount, Virginia. Ten other students of Dairy Science attended the convention and reported that it was a very educational experience.

Kansas State News

Jack Van Horn, a senior in dairy production, won Kansas State College's 1957-58 Borden scholarship. Van Horn will receive $300 from the Borden Foundation, Inc.

Jack Van Horn

Van Horn is an outstanding student at Kansas State, with a near-perfect scholastic record. During his first three years he has maintained a grade average of 2.747 out of a possible 3.000. He is president of the College of Agriculture Student Council, active in college government, and has been a leader in Dairy Club functions at Kansas State.

Van Horn was a member of Kansas State's dairy cattle judging team that won top honors at Waterloo, Iowa, in 1957.

Fall activities at Kansas State college included the annual fall steak fry. A group of 75 Dairy Club members and dairy dpartment staff ate juicy, sizzling steaks and spent the evening square-dancing. This was an excellent opportunity for members to become better acquainted with the college dairy staff.

Kansas State is proud of its thre dairy judging teams. The senior dairy cattle team won first in the college division at Waterloo, Iowa; the junior cattle judging team topped all colleges represented at the International Dairy Show at Chicago. The Dairy production judging team won third at the College Students' International contest at San Francisco, and sixth at International Collegiate Dairy Products Contest in Chicago. Thomas W. Townsend was second high individual at the San Francisco contest. Thirteen of the 14 members on these judging teams are members of the college Dairy Club.

Dairy Technology Club of Ohio State University

On November 2, the students and faculty of the Dairy Technology Department entertained approximately 140 alumni at the 9th annual Dairy Technology Homecoming celebration. Several leaders of the dairy industry and the University were present to enjoy the late morning brunch.

Ron E. Walters, president of the Dairy Technology Club, spoke about the activities of the club and presented the members of the Dairy Technology judging teams to the audience.

The event was highlighted by the presentation of three awards. Karl Kepner, a junior in Dairy Technology, received the "Golden Guernsey Award" for outstanding scholarship. Dr. E. F. Almy, professor in Agricultural Biochemistry, received a "Certificate of Achievement" for 25 years of teaching Dairy Chemistry to Dairy Technology majors. Finally, the "Award of Merit," which has been presented annually by the Department of Dairy Technology for the past nine years for outstanding service to the dairy industry, was presented to Mr. P. L. Haymes, Director of The United Milk Products Company, Cleveland, Ohio. The presentation was made by Dean L. L. Rummell of the College of Agriculture.

Other Important News at O.S.U.

Effective November 14, 1957, two separate chapters of the Student Affiliate Branch of the A.D.S.A. were recognized at Ohio State University. One chapter includes the Dairy Science students and the other the Dairy Technology students.

The two chapters will elect their own officers and conduct their own meetings. Joint social events will be held once each quarter.

Ron E. Walters is president of the new Dairy Technology Club. Other officers include Karl Kepner, vice-president, Richard West, secretary, and Richard Githens, treasurer.

The Dairy Tech Club meets regularly on the second and fourth Thursday of each month. One meeting a month is devoted to business, the other meeting to discussion of topics of interest. At the December meeting a discussion was led on "Milk and Radio-Activity."

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Dairy Science Club of Ohio State University

The Ohio State University student affiliate chapter, under the leadership of president Ron Eberhard, is a wide-awake group, with a broad range of activities.

Autumn quarter saw the club serving meals to dairy science alumni at the department's annual "Brunch" and to the folks attending the Ohio Winter Dairy Day program. Both projects proved to be worthwhile money-making schemes.

Social events take precedence during the winter with a "best foot forward" square dance party in cooperation with the Home Economics Club here at O.S.U. How these dairy majors do enjoy a good fast dance! Don Wagner, social chairman, is in charge of planning the Winter Dinner-Dance, our major social event of the year. This evening is a joint party with the Dairy Technology Club. A banquet-style dinner precedes an after-dinner speaker, presentation of keys to the judging teams, and fiery competition between the clubs in the annual "skit" challenge. Both groups present their most promising talent acting in highly original productions. The winner is awarded possession of the much-coveted and appropriate trophy—a beautiful stainless steel milk bucket. The remainder of the evening is spent in dancing.

The Central Ohio Breeders Association Annual Meeting comes to campus in the near future and brings a big job for A.D.S.A. Jim Miley and associates are working overtime to make arrangements for serving lunch to an expected group of 500 persons.

This year, for the first time, A.D.S.A. will prepare and publish a brochure of the year's activities. Philip Gay is at the reins. Copies of the finished product may be obtained by making request to O.S.U. Department of Dairy Science.

Competition within the club shifts into high gear with the advent of spring. Louis Davis and his boys are already picking cattle for the various classes to be used in the Judging Contest. Class winners, breed winners, as well as grand champion, share a notable array of awards.

Members will try their skill at fitting and showmanship in the Second Buckeye Dairy Show scheduled for April 19. The show is held in conjunction with the Ohio P.D.C.A. Judging Conference and Honor Banquet. Last year's winner, Lois Garbig, as general chairman, directs the very capable list of committee chairmen. Howard Hansen will be in charge of show arrangements; Tom Settlemire, banquet; Jim Price, program, and Jim Deeter, publicity.

Awards will be made to Judging Contest and Dairy Show winners as a feature of the Honor

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Banquet which follows the show. Also, the eagerly anticipated presentations of the "Sprouted Oats" and "Proven Sire Certificate" will be made. Identity of the recipients of these awards is a closely guarded secret until the banquet.

In a more serious and meaningful vein will be the honoring of a worthy individual who has made outstanding contributions to the dairying industry in Ohio.

A rollicking picnic late in the spring will punctuate the year's activities and open the way for new ideas in the coming school year.

Santa Visits L.S.U. Dairy Club's Christmas Party

The annual Christmas party of the Louisiana State University Dairy Science Club was held on the evening of Friday, December 13, at the Nelson Memorial Building. The faculty members of the dairy department and their families, club members and their dates, and guests had a surprise visit from Santa Claus, who was loaded with gifts. The building was appropriately decorated for the Christmas season, and all the young ladies received a corsage. Punch, cookies, fancy sandwiches, and delicacies provided appetizing refreshments throughout the evening. The music for dancing was furnished by a three-hour recording, made at station WLSU, of the best songs to dance by.

Other recent activities of the club have included a decision to publish "The Bayou Dairyman" student annual again this year. This annual would contain pictures and write-ups on the club's activities, information about the dairy department at the university, and encourage high school students to attend Louisiana State University and study agriculture.

The club voted to contribute money to the Dairy Judging Team to help defray some of the expenses the individual members incurred at the various contests. Dr. J. B. Frye, Head of the Dairy Department, read a letter from the Quaker Oats Company commending Bruce Jones for his outstanding ability to judge dairy animals and give reasons for his placings, and for being the top student in judging dairy animals at the International Livestock Show in Chicago.

Georgia Dairy Science Club

On the 26th of April, 4-H and F.F.A. students from more than 75 high schools will once again eagerly flock to the University of Georgia. They will be coming to the 12th annual Dairy Day, which is sponsored by the Dairy Science Club, a student affiliate of the American Dairy Science Association.

Dairy Day serves many purposes, among the more important being to give club members experience in leadership and organization. For instance, much of the responsibility for the sue-

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cess of the day falls upon Jim Lee, this year's Dairy Day chairman. Another important purpose of the event is to provide high school students interested in dairying with an opportunity to come to the University and participate in the Dairy Day judging contest.

Highlighting the activities of the afternoon is the three-breed heifer sale, which obtains high quality heifers from Georgia herds for 4-H and F.F.A. activities. This sale, entirely organized and managed by dairy students, is the first instance in this country of a club undertaking such an activity, and the only time that the three breed organizations have consented to a sale together, using the same auctioneer.

The final and most lasting benefit derived from Dairy Day is realized when the income to the club from this activity is placed in a scholarship fund for dairy students who might not attend school otherwise.

FROM THE SECRETARY'S DESK

An Analysis of Our Association

Is our type of membership becoming too broad?
Why do we publish a scientific journal?
What about a separate, "popular" journal?
Do we need advertising in the JOURNAL?
Why are there not more manufacturing papers in the JOURNAL?
Why don't more Industry members attend the Annual Meeting?
Are we as democratic as we could be?

These and other questions are sometimes raised. It is desirable to state some facts concerning our Association, in order that the membership can understand the various activities of the Association and the work of its Board of Directors. Your Secretary is, perhaps, as well qualified as anyone to make such an analysis, because he has been a member for many years—first as a teacher and as a businessman, and then as Secretary-Treasurer. He has also been through the various offices in the Association. Therefore, he feels competent to present an unbiased version to the Association of its various activities and problems.

Membership

A letter was received from a member who commented on the membership campaign in his state as follows:

"Having complied with the material and instructions, we haven't received any new members that I know about. It seems to me that we must concentrate our efforts on those who are engaged in teaching, research, and extension activities. Most of the people in industry can't interpret the articles that are published in the JOURNAL OF DAIRY SCIENCE, with some exceptions. They are more interested in their various trade associations, dairy technology societies, and similar organizations.

"I have always felt that the American Dairy Science Association should remain the professional organization of those engaged in research, teaching, and extension, including people in industry who have similar interests. I can not become enthused about trying to get people to join the organization who have no interest in it, for the purpose of increasing membership and the JOURNAL'S circulation.

"Therefore, I am not quite as enthused as I should be about trying to secure members from all phases of the dairy industry. It seems to me that our association should try to stand on its own feet financially, limit the membership to those who are really interested, and do everything in our power to maintain its high level of professionalism."

This dairy scientist may be interested in some facts about our membership. To indicate differences in viewpoints, the membership committee chairman in an adjoining state has secured 19 new members.

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"I can say with all sincerity that this is the organization in which I am most interested of all the ones to which I belong. In fact, it is easiest to part with the dues for this organization."

Let us look at our constitutional membership requirement. It states:

"Membership is open to those who have had technical college training in dairying or in allied fields, or are filling responsible positions of a professional character requiring a technical knowledge of dairying."

This requirement is adhered to, but it leaves room for variations in training and position. I have studied the applications of 106 new members and find that practically all of them are college graduates with one or more degrees, although a college degree is not required in the membership statement. Our membership committees are certainly using good judgment. Professional background, as well as education, must be considered. The following random tabulation illustrates the positions held by members and lists their degrees, if any:

College training and present position

Ph.D.
- Technologist in a flavor laboratory.

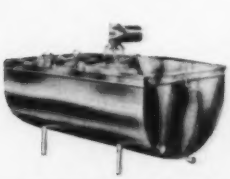
D.V.M.
- Veterinarian

M.S.; Sc.D. (Honorary)
- Director of research for a milling company.

B.S.
- Manager of a butter warehouse.
- Manager of an artificial breeders' corporation.
- Soil conservationist, Bureau of Indian Affairs.
- Salesman for an equipment company.
- Manager of a dairy company.
- Fieldman for a dairy association.
- Former city health inspector, now owns company.
- Owner of 3,000 acres devoted to dairy cattle.
- Director of research for large company serving dairy industry.

A.B.
- Manager of laboratories of large dairy company.

Certificate of Business Administration
- Ten years as general manager of a dairy cooperative.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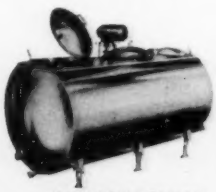
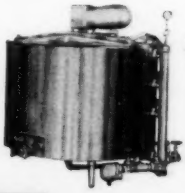

VACUUM BULK COOLERS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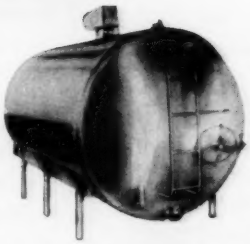
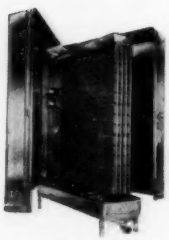

COLD WALL TANKS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Manager of a milk products association.

No degree

Forty years of experience in ice cream industry, now owner and manager. Member of a state board of agriculture.

Twenty-eight years of experience, now executive vice-president and manager of a dairy company.

Ten years of experience in field service in quality control.

I agree that we should not try to get uninterested people to join the A.D.S.A., in order to increase membership. The tabulated information indicates that within the constitution there are older, as well as younger people, and graduates of various colleges, as well as those who have advanced far through experience, who have become members of the Association. Incidentally, out of 106 applications only 20 came from universities. This is owing to the fact that most of these people already belong to the A.D.S.A. This leads us to inquire about the composition of our present membership:

The 1956 membership showed 42.3% in teaching, research, or extension at the universities, 33% in commercial dairy plants, and the remainder in 19 other categories, ranging from 5.2% in the dairy equipment and supply industry, to one housewife (0.05%). A further breakdown of the university dairy staffs showed 20.2% in teaching, 14.8% in research, 41.3% in teaching and research, 21% in extension, and small percentages in combinations of teaching, research, and extension. Production work comprised 64.5%, as against 33.5% in manufacturing. The Association membership is divided about equally between manufacturing and production. Because the dairy industry has so many specializations, it seems logical that we should have broad membership requirements. It appears that we should grow gradually, rather than rapidly, and stay within our present constitution.

The Journal

The questions raised at the outset concerning the JOURNAL can be answered in one statement which, it will be clearly seen, has a definite relationship to our membership.

It is natural to researchers that the Association should publish a highly scientific journal. To some members in industry the desire is for information that readily can be put to use. Some would prefer to have a JOURNAL that contains only scientific papers, with little or no advertising. A "popular" journal, to go along with a scientific journal, has been considered by the Association. It is obvious that the type of

journal must be determined by the composition of the membership and the state of the finances. The cost of the JOURNAL is $60,000 a year. Income from membership and affiliate member dues and from subscriptions in 1956 amounted to $44,000, leaving a deficit of $16,000, plus another $16,000 for association operating expenses. Dividing $32,000 by a membership of 2,000 results in a figure of $16 which, if added to the present dues of $10, would require dues of $26 per member to operate the JOURNAL and Association activities, without advertising and sale of reprints. Net income from the sale of reprints in 1956 was $6,232. It is presumed that this income might accrue from a strictly scientific type of journal. Even though we credit this amount, it would require dues of $22.85 to run the Association and publish the JOURNAL. Actually, the situation would be worse if members were lost and replacements were not found. *As the number of journals printed decreases, the cost per copy rises.* It costs just as much to set the type for a journal of a certain number of pages, regardless of the number of copies printed. As a matter of fact, as the JOURNAL is now being printed, an extra 100 copies a month costs about $44. It was only because of our income from advertising that we finished with a profit in 1956.

While it is true that the American Chemical Society, The Food Technologists, and others publish both scientific and popular journals, it must be remembered that their membership is large, enabling them to do things that we can not do. A committee of our Association has studied this problem and concluded that the type of journal we are now publishing serves our needs best. The dues are reasonable and it is doubtful if an increase would be welcomed by the membership. There are more production than manufacturing papers published in the JOURNAL and this can be explained, in part, by the fact that there are about twice as many researchers in production as there are in manufacturing in the colleges and universities. However, industry members are beginning to submit more papers to the JOURNAL. The papers in the Our Industry Today section of the JOURNAL are of interest both to the manufacturing and to the production groups. Incidentally, it may be of interest to note the publisher's circulation report covering the October, 1957, issue:

Members	2,291
Subscribers	1,622
Student affiliates	754
Copies to advertisers in current issue	42
Complimentary	5
Life	11
Honorary	13
Charter	2
Total	4,740

Attendance at Annual Meetings

I attended the meeting of the Program Committee that is planning the 1958 meeting of our Association and my sympathies are with them. A large number of papers will be presented to the committee for consideration, written by authors ranging from experienced researchers to graduate students.

The committee must consider the membership and the interest there will be in these papers; then, they must prepare an integrated program. As has been the case with the JOURNAL, if we consider both the production and extension sections, more papers are presented in these areas than in manufacturing. The papers in manufacturing have become more highly technical during the past 25 years. It is true that fundamental research is essential to the future of any industry, and this is appreciated by our commercial dairy people, though they do not attend our annual meeting in large numbers. At the 1957 meeting at Oklahoma State University, only 66 members from the Dairy Industry and 45 from the equipment and supply companies were registered, out of a total of 629. Undoubtedly, we need to do a better job in explaining the application of results presented in scientific papers, and to put more effort on having more papers and symposia of interest to industry members, at our annual meeting. An effort will be made to do this at the 1958 meeting.

Are We a Democratic Organization?

The Secretary's files contain lists of committees that have served the Association for the last five to ten years, varying for different committees. A study shows that 230 different members have served on these committees and that 763 years of service are represented. In addition, 104 others have served as various officers of the association or its sections. These figures certainly indicate democracy in action and a willingness to serve. The annual business meeting of the Association now, completely and concisely, advises the membership of the actions of its officers and committees and presents an opportunity for any and all to comment. Furthermore, those who have attended annual meetings certainly must realize the friendly, democratic spirit that prevails in the various meetings, in the corridors, lounges, eating places, and on the campus. It is one grand family party. Therefore, it would seem that there is no reason to change the present status of our Association or of the JOURNAL OF DAIRY SCIENCE.

—H. F. JUDKINS

When accompanied by adequate amounts of all essential nutrients . . . fats play an important role in nutrition by furnishing a concentrated source of energy for carrying out daily activities.

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43.2% from fats and oils (excluding butter) . . .
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2.7% from dry beans, peas, nuts and soya flour . . .
1.4% from flour and cereal products . . .
0.7% from vegetables and fruits . . . and
5.4% from other foods.

Of the calories consumed by Americans:

⅓ comes from animal food products (all dairy foods including butter, meat, poultry, fish and eggs) which supply: ¾ of our calcium; ⅔ of our riboflavin; about ⅔ of our protein; ½ of our niacin; ⅓ of our iron and thiamine; and ¼ of our vitamin A, chiefly preformed . . .

⅓ comes from plant food products (dry beans, peas, nuts, flour, cereal, fruits and vegetables) which supply: 9/10 of our ascorbic acid; almost ⅔ of our vitamin A and thiamine; about ½ of our iron and niacin; about ⅓ of our riboflavin; and ¼ of our calcium . . . and

⅓ comes from concentrated fat and carbohydrate foods (fats and oils, without butter, sugars and sirups) which provide less than 1/20 of any essential nutrient for which allowances are established.

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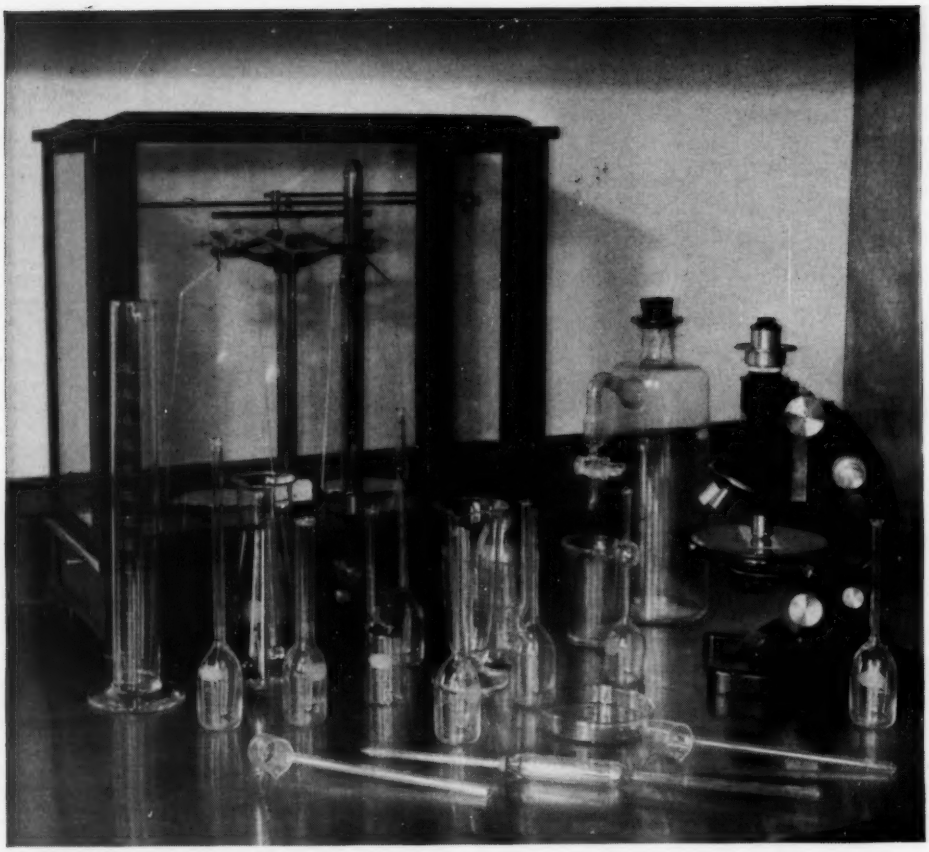

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CALCIUM ION CONCENTRATION IN MILK

H. TESSIER AND DYSON ROSE

Division of Applied Biology, National Research Laboratories, Ottawa, Canada

SUMMARY

The murexide method of Smeets *(15)*, modified to give increased sensitivity and coupled with a pressure ultrafiltration technique that provides sufficient sample in approximately 1 hr., is a simple, convenient method for the determination of calcium ion in milk on a semiroutine basis. With suitable adjustment of the ionic strength and sodium content of the reference solutions, the method is applicable also to concentrated milk products.

Skimmilk was found to contain from 2.5 to 3.4 m*M*/liter Ca^{++}. Addition of calcium to milk precipitated both phosphate and citrate but increased $[Ca^{++}]$; addition of phosphate precipitated calcium and decreased $[Ca^{++}]$, and addition of citrate dissolved colloidal phosphate but decreased $[Ca^{++}]$. The apparent solubility products of di- and tricalcium phosphate in milk appear to be approximately 1.5×10^{-5} and 1×10^{-24}, respectively.

Heating skimmilk caused precipitation of calcium phosphate and lowered $[Ca^{++}]$. Concentration of milk also caused precipitation of calcium phosphate, but $[Ca^{++}]$ increased.

Although the relations between calcium ion concentration ($[Ca^{++}]$) and other properties of milk are not obvious *(3, 6)*, $[Ca^{++}]$ is probably one of the factors controlling the characteristics of milk colloids. $[Ca^{++}]$ must also be related to the solubility of the calcium phosphate salts present in the milk colloids. Several methods for the estimation of calcium ion concentration in milk have been published recently *(1, 3, 6, 14, 15)*. Of these, the murexide method *(15)* appeared to be the most simple and direct, but this method required some modification before it could be applied to concentrated milk products. Therefore, a modified method was developed, together with a rapid ultrafiltration procedure. Details of these methods, and additional data on the relation between $[Ca^{++}]$ and total calcium, phosphate, and citrate are presented.

MATERIALS AND METHODS

Morning milk was separated raw at about 34° C., and the skimmilk pasteurized at 66° C. for 30 min. Ultrafiltrates were prepared from 100-ml. lots of milk at room temperature by filtration at 30 p.s.i.g. through 1-in. diameter dialysis tubing (Cellophane) supported by stainless steel screens (Figure 1). Ultrafiltrate was collected in a large test-tube which totally enclosed the screen so as to reduce evaporation. Approximately 10 ml. of ultrafiltrate were collected per hour.

For the determination of ionic calcium all glassware was rinsed in 1:40 hydrochloric acid. Standard solutions were prepared in glass-distilled water and stored in polyethylene bottles. Murexide (ammonium purpurate, Eastman

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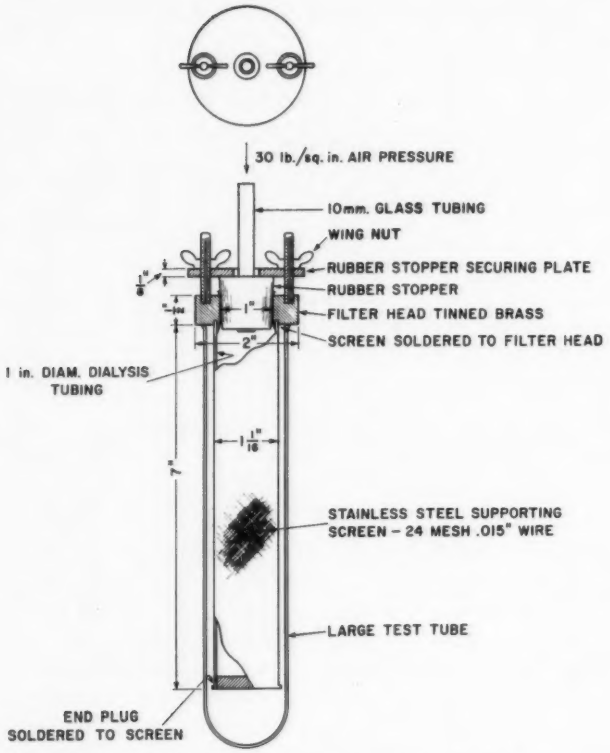
30 lb./sq. in. AIR PRESSURE
10mm. GLASS TUBING
WING NUT
RUBBER STOPPER SECURING PLATE
RUBBER STOPPER
FILTER HEAD TINNED BRASS
SCREEN SOLDERED TO FILTER HEAD
1 in. DIAM. DIALYSIS TUBING
STAINLESS STEEL SUPPORTING SCREEN – 24 MESH .015" WIRE
LARGE TEST TUBE
END PLUG SOLDERED TO SCREEN

FIG. 1. Apparatus for rapid ultrafiltration of milk.

Kodak Co.), prepared as a 0.1% solution in water, was stable for several days in polyethylene at 4° C. Standard calcium solutions were prepared from reagent grade calcium carbonate and a slight excess of hydrochloric acid, with subsequent neutralization to pH 6.8 with potassium hydroxide.

The procedure finally adopted for the determination of $[Ca^{++}]$ was to place 0.1 ml. of murexide solution in a test-tube, add 4 ml. of milk ultrafiltrate, and determine the absorption at 520 and 480 mμ with a Carry[2] recording spectrophotometer. A second aliquot of ultrafiltrate served as the reference blank. Calcium standards at appropriate potassium : sodium ratios and ionic strength were prepared at the same time, and the calcium content of the ultrafiltrate estimated from a plot of the $E'_{520} - E'_{480}$ (vide infra) values. Readings could be made immediately after addition of the samples or delayed up to 2 hr.

Total calcium was determined by a turbidimetric potassium oleate method *(7)*, phosphate by a colorimetric phosphomolybdate method *(8)*, and citrate by a

[2] An Evelyn colorimeter can be substituted for the recording spectrograph by adding 10 ml. of sample to 0.1 ml. of murexide and reading with No. 515 and 470 filters.

modification of the acetic anhydride-pyridine method *(11)*, in which trichloracetic acid was replaced by 0.1 *N* hydrochloric acid.

The concentration of specific salts in milk was increased by adding up to 1.5% by volume of molar solution of calcium chloride (neutralized to pH 6.7 with potassium hydroxide), or potassium phosphate (pH 6.7) or sodium citrate (pH 6.8) to the milk. The dilution of each sample, including the control, was kept constant by appropriate additions of distilled water.

DEVELOPMENT OF THE CALCIUM ION METHOD

The absorption maximum of a murexide solution changes progressively from 520 to 480 mμ as the [Ca^{++}] is increased *(12, 15)*. Rafflaub *(10)* estimated calcium ion concentration from the extinction coefficient of 470 mμ with reference to standard calcium solutions. Smeets *(15)*, following Schwarzenbach and Gysling *(12)*, determined an "average dissociation constant" and calculated [Ca^{++}] of unknown solutions by means of this factor. To determine the most suitable procedure, the whole absorption curve was recorded for each solution and various methods of relating absorption readings to [Ca^{++}] were tried. A plot of the absorption at a single wavelength against [Ca^{++}] gave curves that were relatively flat at calcium levels above 2 m*M*/liter (Figure 2). A plot of wavelength of the absorption maximum against [Ca^{++}] gave a similar curve, but had the advantage of being independent of murexide concentration. Increased sensitivity was obtained by plotting the value obtained by subtracting the ab-

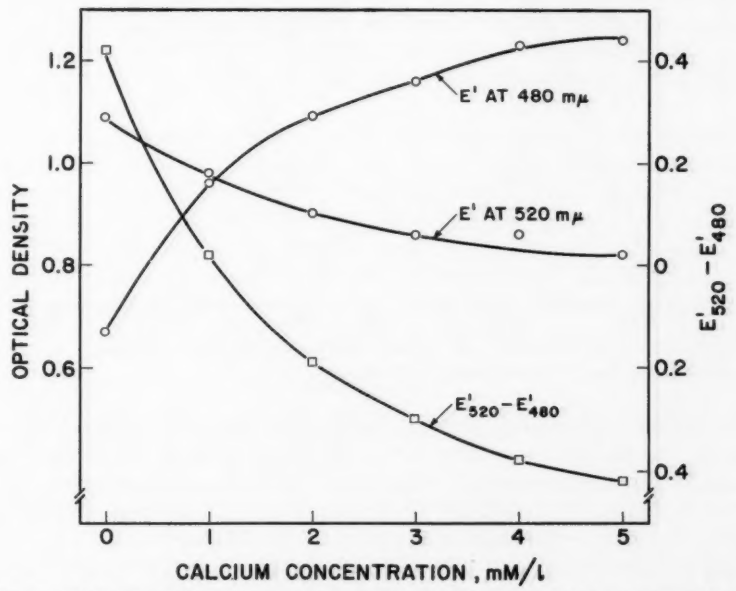

FIG. 2. Light absorption of murexide at various calcium levels (0.1 ml. murexide, 4 ml. calcium solution, $\mu < 0.01$).

sorption at 480 mμ from that at 520 mμ ($E'_{520} - E'_{480}$ value) against [Ca^{++}] (Figure 2), and at murexide concentrations of about 0.1 mgm/4 ml. sample this curve was only slightly affected by murexide concentration. It has, therefore, been used throughout this work.

In agreement with the results of Smeets *(15)*, lactose and magnesium did not interfere with the estimation of [Ca^{++}], citrate reduced [Ca^{++}] in accordance with the known dissociation constant *(4)*, and phosphate reduced it slightly. Therefore, no further study of these factors was undertaken.

On the other hand, ionic strength and sodium ions markedly affected the results. Increasing ionic strength with potassium chloride had only a moderate effect on the absorption of murexide in the absence of calcium but, in the presence of calcium, increasing the potassium shifted the absorption curve of calcium murexide towards that of murexide alone (Figure 3). This effect persisted throughout the range of measurable calcium concentrations.

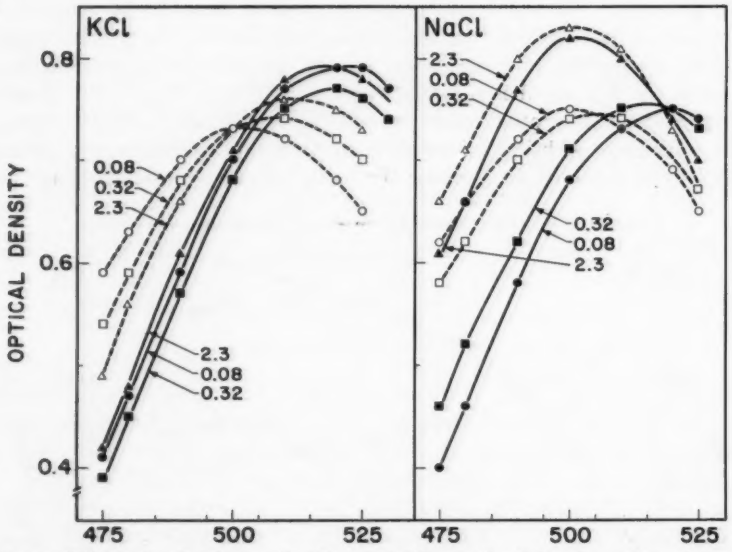

FIG. 3. Effect of potassium and sodium chloride on the light absorption of murexide. Solid lines—no calcium; dotted lines—2 m*M* calcium.

In contrast to potassium, sodium affected the absorption of murexide itself, increasing the absorption maximum and shifting it towards lower wavelengths (Figure 3). In the presence of calcium, sodium had a complex effect, the absorption at 480 mμ, for example, being decreased by increasing sodium up to 0.32 *M*, but being increased again by 1.0 or 2.3 *M* sodium.

Replacing potassium chloride with sodium chloride at fixed ionic strength (Table 1) had only a small effect at μ=0.08, but the differences became progressively greater at higher ionic strengths. Standard curves prepared in the

TABLE 1

Effect of potassium-to-sodium ratio on the light absorption of murexide
(2 m*M* calcium in all samples)

K:Na Ratio	$E'_{530}-E'_{450}$ at ionic strength			
	0.08	*0.32*	*1.0*	*2.3*
All K	+0.05	+0.13	+0.18	+0.23
3K:1Na	+0.04	+0.12	+0.15	+0.15
1K:1Na	+0.03	+0.10	+0.12	+0.10
1K:3Na	+0.03	+0.08	+0.09	+0.05
All Na	+0.03	+0.08	+0.06	−0.01

presence of potassium alone are thus acceptable for work with normal milks, but the potassium-to-sodium ratio found in the samples must be approximated in the standard solutions when [Ca^{++}] is determined in concentrated milk products.

RESULTS AND DISCUSSION

Application of the original method *(15)* and our modified method to ultrafiltrates from normal milks gave values that did not differ by more than 0.1 m*M* Ca^{++}/liter. Reproducibility of results obtained with the modified method appeared to be approximately ±0.05 m*M* in normal milks and ±0.1 m*M* at higher calcium levels. Determination of [Ca^{++}] in a synthetic mixture resembling protein-free milk serum gave a value of 2.2 m*M*, whereas calculation from published dissociation constants for calcium citrate *(4)* gave a value of 2.31 m*M*.

Values for a series of skimmilks tested over a 2-yr. period ranged from 2.5 to 3.4 m*M* Ca^{++}/liter. This range is slightly higher than those given by van Kreveld and van Minnen *(6)* and by Christianson *et al. (3)*, but agrees with that of Smeets *(15)* and is lower than the values reported by Affsprung and Gehrke *(1)*.

The gradually increasing concentration of milk colloids within the membrane during ultrafiltration did not detectably affect [Ca^{++}] in three consecutive 10-ml. samples of ultrafiltrate taken from one 100-ml. aliquot of milk. Whole and skimmilk yielded ultrafiltrates with the same [Ca^{++}], thus confirming the results of van Kreveld and van Minnen *(6)*.

Addition of calcium to skimmilk before ultrafiltration caused an increase in [Ca^{++}] and total calcium, and a decrease in total phosphate and citrate in the ultrafiltrate (Table 2). Smeets *(15)* observed a similar decrease in total phos-

TABLE 2

Effect of the addition of calcium to skimmilk on composition of the ultrafiltrate
(All samples adjusted to pH 6.57 after addition of calcium)

Calcium added	[Ca^{++}]	Total Ca	Total PO_4	Citrate	$[Ca^{++}][HPO_4^{=}]$	$[Ca^{++}]^3[PO_4^{\equiv}]^2$
	(mM/liter)					
0	3.4	9.9	9.9	10.5	1.3×10^{-5}	4.3×10^{-22}
2.5	4.1	10.3	8.9	10.2	1.4×10^{-5}	6.1×10^{-22}
5.0	5.3	11.7	7.8	10.1	1.6×10^{-5}	10.1×10^{-22}
7.5	6.5	11.6	7.0	9.6	1.8×10^{-5}	15.1×10^{-22}
10.0	7.2	11.5	5.7	9.2	1.6×10^{-5}	13.5×10^{-22}

phate and, on the assumption that only dicalcium phosphate precipitated, calculated the "change in protein-bound calcium." The data (Table 2) show that citrate also decreased, but because tricalcium phosphate also may have precipitated, calculation of protein-bound calcium is not possible.

Addition of phosphate to skimmilk decreased [Ca^{++}] and total calcium in the ultrafiltrate (Table 3), and addition of citrate to skimmilk decreased [Ca^{++}] and increased total calcium, total phosphate, and pH of the ultrafiltrate (Table 4).

TABLE 3

Effect of the addition of phosphate to skimmilk on composition of the ultrafiltrate
(All samples adjusted to pH 6.62 after addition of phosphate)

Phosphate added	[Ca^{++}]	Total Ca	Total PO_4	[Ca^{++}][$HPO_4^{=}$]	[Ca^{++}][$PO_4^{\equiv}$]^2
		(mM/liter)			
0	2.8	9.2	9.6	1.1×10^{-5}	3.3×10^{-23}
5	2.5	8.9	14.1	1.5×10^{-5}	5.1×10^{-23}
10	2.3	8.2	19.5	1.9×10^{-5}	7.4×13^{-23}
15	2.1	7.4	23.2	2.0×10^{-5}	8.0×10^{-23}

TABLE 4

Effect of the addition of citrate to skimmilk on composition of the ultrafiltrate

Citrate added	pH of ultrafiltrate	[Ca^{++}]	Total Ca	Total PO_4	[Ca^{++}][$HPO_4^{=}$]	[Ca^{++}]^3[$PO_4^{\equiv}$]^2
(mM/liter)			*(mM/liter)*			
0	6.72	2.4	7.8	9.0	1.0×10^{-5}	3.7×10^{-23}
5	6.82	1.9	9.8	10.2	1.0×10^{-5}	4.7×10^{-23}
10	6.90	1.8	10.9	11.1	1.1×10^{-5}	8.1×10^{-23}
15	7.02	1.8	13.1	12.8	1.5×10^{-5}	23.2×10^{-23}

The calculated values for [Ca^{++}] [$HPO_4^{=}$] (k_2) and [Ca^{++}]^3 [$PO_4^{\equiv}$]^2 (k_3), assuming all of the phosphate in ionic form,[3] are given (Tables 2, 3, and 4). The average value of k_2 in the initial samples (skimmilk plus 1.0 or 1.5 ml. of water per 100 ml.) is 1.1×10^{-5}, and the range for all samples, regardless of calcium, phosphate, or citrate addition is from 1.0 to 2.0×10^{-5}. The average value for k_3 in the initial samples is 3.8×10^{-23} (note the higher initial value in Table 6), and the range for all samples is from 3.3 to 23.2×10^{-23}. Precipitation of calcium salts occurred in samples with added calcium or phosphate, whereas solution of colloidal phosphate occurred in the samples with added citrate; therefore, it can be assumed that most of these samples were saturated with calcium phosphate. The variability of both k_2 and k_3 corresponds approximately to a two-fold variation in [Ca^{++}] at fixed phosphate concentration and pH; therefore,

[3] Dissociation constants of $K_1^1 = 1.154 \times 10^{-2}$, $K_2^1 = 1.68 \times 10^{-7}$, and $K_3^1 = 2.34 \times 10^{-12}$ were calculated from the equation of Hentola (*Chem. Abstr.*, **44**: 5192i. 1950.) for a potassium chloride solution of ionic strength 0.08. These data were selected because they give all three constants under a single set of conditions approximating those of milk. The K_2^1 value is also in good agreement with that obtained in the more extensive work of Bates and Aeree (*J. Research Natl. Bur. Standards*, **34**: 373-394. 1945).

no conclusion can be drawn regarding the nature of the precipitated salt. Errors involved in estimating the composition of precipitated salt by difference are too great for the calculation to be significant. However, the data do suggest that the solubility products for di- and tricalcium phosphate in milk are roughly 1.5×10^{-5} and 1×10^{-24}, respectively. These values are much higher than those reported under other conditions *(5)*, but Boulet and Rose *(2)* have pointed out that published values can not be applied to milk.

Heating skimmilk to 66° C. for 30 min. lowered total calcium and phosphate in the ultrafiltrate obtained approximately 1 hr. after recooling to room temperature, but [Ca^{++}] did not change significantly (Table 5). Heating to 82° C. for

TABLE 5

Effect of heating milk and ultrafiltrate on composition of the ultrafiltrate

Treatment	pH	[Ca^{++}]	Total Ca	Total PO_4	Total citrate
		(mM/liter)			
Milk heated before ultrafiltration					
Control	6.79	2.6	8.6	9.9	7.8
66° C., 30 min.	6.72	2.7	7.5	9.2	7.7
82° C., 30 min.	6.70	2.4	6.8	8.5	7.7
Ultrafiltrate heated [a]					
Control	6.63	2.9	7.1	10.3	7.6
66° C., 30 min.[b]	6.59	2.5	7.0	10.3	7.4
82° C., 30 min.[b]	6.36	1.6	5.2	8.9	7.4

[a] The ultrafiltrate was not prepared from the same milk as used in the "Milk" part of the table.

[b] Centrifuged at 28,000 r.p.m., No. 30 head, Spinco preparatory centrifuge, to remove finely dispersed precipitate.

30 min. lowered total calcium and phosphate still further and lowered [Ca^{++}] by 0.2 m*M*/liter. Van Kreveld and van Minnen *(6)* reported similar changes after heating to 95° C. for 3 min., and Christianson *et al. (3)* report a 0.6 m*M* loss after heating to 85° C. for 30 min.

Heating the ultrafiltrate itself caused the formation of a finely dispersed precipitate and a decrease in [Ca^{++}] (Table 5). Removal of the finely dispersed precipitate by high-speed centrifugation (66,000 × G for 1 hr.) decreased total calcium and phosphate without further changes in [Ca^{++}].

Concentration of skimmilk by low-temperature evaporation (21° C.) increased [Ca^{++}] proportionately less than it increased total calcium or phosphate (e.g., [Ca^{++}] in 3:1 milk—4.4 m*M*/liter, total calcium 23.2 m*M*/liter, and total phosphate 28.8 m*M*/liter. Concentration of the ultrafiltrate itself, followed by centrifugation to remove precipitated salts and lactose, decreased pH and increased the concentration of all salts (Table 6). Citrate concentration increased essentially to the same extent as sodium or potassium, indicating that no calcium citrate precipitated. Calcium phosphate, on the other hand, precipitated quite extensively, although the solubility of these salts as indicated by [Ca^{++}] [$HPO_4^{\equiv}$] and [Ca^{++}]^3 [$PO_4^{\equiv}$]^2 was increased more than tenfold by the increasing ionic strength.

TABLE 6

Effect of concentrating an ultrafiltrate

Approximate concentration	pH	$[Ca^{++}]$	Total Ca	Total PO_4	Citrate	Potassium	Sodium	$[Ca^{++}][HPO_4]$	$[Ca^{++}]^3[PO_4\equiv]^2$
		(mM/liter)							
Normal	6.71	2.8	7.8	11.2	8.9	37.8	16.5	1.5×10^{-5}	8.5×10^{-29}
3.6:1	6.26	6.4	28.0	37.0	32.0	134.0	61.0	5.5×10^{-5}	36.0×10^{-29}
11:1	5.84	15.4	59.0	100.0	94.0	405.0	183.0	15.6×10^{-5}	104.0×10^{-29}

ACKNOWLEDGMENTS

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HARDNESS OF BUTTER. I. INFLUENCE OF SEASON AND MANUFACTURING METHOD[1]

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SUMMARY

Hardness was measured on weekly samples of conventionally churned and continuously made butter,[2] taken over a period of 1 yr. Penetrometer and sectility methods were used at 17 and 12° C., respectively. The continuously made butters were consistently harder than the conventionally churned butters. Similar seasonal variations were found for both butters and were greater than differences between the two butter types at 17° C. Hardness maxima occurred simultaneously with softening-point minima, as determined previously. Hardness of conventional butter was less influenced by the experimental temperature difference than was that of the continuous type butter. The hardness of the continuous type butter decreased as gas was added and, therefore, gas content may be a partial cause of the hardness differences between the two butter types. A major portion of the setting of the continuous butter occurred during the first 3 hr. after manufacture and was attributed to crystallization, which was still in progress. The hardening taking place after this first period was attributed to thixotropic changes.

Extensive hardness increase, assumed to be caused by thixotropic changes, occurred in conventional butter and extended over approximately 1 wk. Setting was prevented in both butters during storage at low temperature (−20° C.), but resumed a normal course on subsequent removal of the butter to higher storage temperatures. A permanent hardness decrease was imparted to both butter types by storage at 20-22.5° C.

It has been shown that butterfat in this region is subject to considerable seasonal variation in chemical composition *(13)*, and it is a well-known fact that such changes will profoundly influence the hardness of butter. In a preliminary investigation, continuously made butter was found to be harder than conventional butter *(14)*. The difference was attributed to the remarkable dissimilarity in crystalline structure, but differences in gas content of these two types of butter also may be partly responsible.

Conventional butter increases considerably in hardness subsequent to removal from the churn. This phenomenon is generally called setting, and has been attributed to thixotropic changes. When a study is made of the influence of manufacturing method on hardness, the setting should be considered an important factor. Storage temperature of the finished butter is also important as a factor influencing the course of hardness changes and should, therefore, receive attention.

The present report is a study of the influence of season and manufacturing method on the hardness of butter. The manufacturing methods chosen were the conventional churning and the fundamentally different continuous method.

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[1] Financial assistance for this research was provided by the Canada Department of Agriculture, Ottawa, Canada.

[2] Made by a process known as Gold 'n Flow, which is copyrighted by the Cherry-Burrell Corp., Chicago, Illinois.

METHODS

Hardness was measured either by the penetrometer method at 17° C. or by the sectility method at 12° C. The penetrometer method was a modification of the procedure described by Kruisheer and den Herder *(7)*. The disk plunger of 4 cm.^{2} surface area was mounted in an apparatus similar to the Swift consistometer described by Clardy *et al. (1)* and moved at 5 cm/min. Each butter sample was prepared for hardness measurement immediately after manufacture, by being pressed into a square frame of 5-cm. side and 2.9-cm. height, fabricated from a 20-gauge stainless steel sheet. For certain experiments, it was necessary to use continuous type butter from the experimental continuous butter-making machine developed in this Department *(15)*, and for this purpose the butter was extruded directly into 2.9-cm. lengths of 2-in. O.D. stainless steel tubing.

At lower temperatures, the resistance to penetration exceeded the range that could be recorded by the gauge of the apparatus, and a sectility method was substituted. This was achieved by replacement of the disk by a taut stainless steel wire (diameter 0.015 in.) held in a frame. The gauge reading was taken when the wire had travelled 2.5 cm. through a block of butter which had first been pushed from its frame.

The butter samples, unless otherwise stated, were kept in the frames at 5-10° C. for 1 wk., then tempered in a water bath for 24 hr. prior to testing. Water bath temperatures of 17° C. in the penetrometer method and 12° C. in the sectility method were maintained thermostatically within ±0.05° C., and measurements immediately followed removal of the samples from the water bath, in a room having a temperature in the range 5-10° C.

Each hardness value reported, unless otherwise specified, is the average of two determinations. Penetrometer hardness is expressed as kg/4 cm.^{2}, whereas sectility is expressed as kg/5 cm., since 5 cm. was the length of the wire in contact with the butter.

RESULTS

Accuracy of methods. To test the accuracy of the methods, ten replicate samples of continuously made and ten of conventional butter were measured by both penetrometer and sectility methods. From the results (Table 1), it appears that the accuracy of the methods is very satisfactory.

TABLE 1
The accuracy of hardness measurements

	Conventional butter		Continuous butter	
	Penetrometer method	Sectility method	Penetrometer method	Sectility method
Range [a]	2.00 − 2.12	0.24 − 0.26	3.94 − 4.14	1.02 − 1.08
Mean	2.05	0.25	4.05	1.05
Av. deviation from mean	± 1.5%	± 4.0%	± 1.4%	± 2.1%

[a] Ten replicate samples of each type of butter.

Round frames gave very slightly higher penetrometer hardness than square frames when the butter was hard, but not when the butter was soft (Table 2).

TABLE 2

Influence of shape of frames on butter hardness values

Description of sample	Penetrometer hardness (*Kg/4 cm.²*)	
	Round frames	Square frames
Hard butter [a]	2.40	2.20
Soft butter [a]	0.84	0.84

[a] Average of four samples each.

Seasonal variation. For a period of 1 yr., butter samples were collected weekly from two local creameries. One manufactured butter by the continuous method and the other by the conventional method. The monthly averages of hardness of each butter type at two temperatures are given (Figure 1). It is to be noted that the butter types follow the same general hardness trend throughout the year (for the penetrometer hardness data, r = .81, significant at the 1% level). This indicates that the difference in physical structure is responsible for

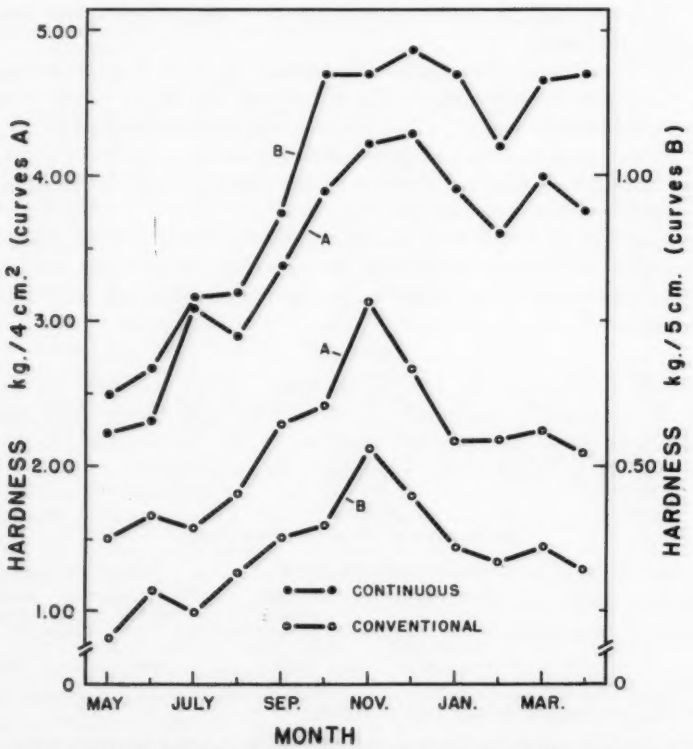

FIG. 1. Comparative hardness of continuous and conventional butter. Curves A: penetrometer method. Curves B: sectility method.

the increased hardness found in the continuous butter, as previous work *(13)* established that the chemical composition of the butterfat of these two Edmonton creameries did not differ significantly. The maximum hardness of both butters occurred in November-December, when the softening point of the butterfat of this region is at maximum value *(13)*; and, conversely, minimum hardness of the butter and minimum softening point of the butterfat coincided seasonally.

The penetrometer and sectility values show a high correlation (for conventional butter, $r = .98$, and for continuous butter, $r = .95$, both significant at the 1% level).

Comparative hardness. It is evident (Figure 1) that on any sampling date the continuously made butter was harder than the conventional butter. The difference was found to be significant at the 1% level.

A remarkable further difference in the hardness properties is revealed in Figure 1. When a point of equal hardness is chosen on the penetrometer curves of each type of butter, it is found that the corresponding sectility values are widely different. The sectility hardness value for conventional butter is then seen to be considerably lower. Thus, the hardness of conventional butter appears to be less influenced by these temperature differences than is the hardness of the continuous butter, since sectility was measured at a lower temperature than was penetrometer hardness.

Influence of gas content. In a preliminary study of the influence of the gas content of butter, samples of continuous type butter with varying nitrogen content were prepared with the experimental continuous machine. The presence of the gas materially lowered hardness values (Table 3).

TABLE 3

Influence of gas content on hardness of experimental continuous type butter

Sample	Gas content	Penetrometer hardness
(No.)	*(%)*	*(Kg/4 cm.²)*
1	0	2.60
	2.0	2.40
	3.6	2.02
2	0	2.48
	1.0	2.28
	16.0	1.68
3	0	2.76
	1.2	2.76
	4.0	1.88
	17.5	1.88

The incidental observation was made, as reported elsewhere *(2)*, that stickiness became apparent in the continuous butter as gas content was increased. The differences in gas content of continuous and conventional butter may explain the differing stickiness of the two butter types.

Setting. A sample of each butter type, taken immediately after completion of manufacture, was maintained in a constant temperature water bath for eight days at the initial temperature, i.e., 5° C. for continuous butter and 12.5° C.

for conventional butter. In this way, the influence of temperature changes on hardness was eliminated. Hardness was measured at these temperatures hourly for 6 hr. and daily thereafter.

Sectility hardness of butter made by the continuous method increased rapidly for the first 6 hr. and less rapidly thereafter (Figure 2). To measure temperature

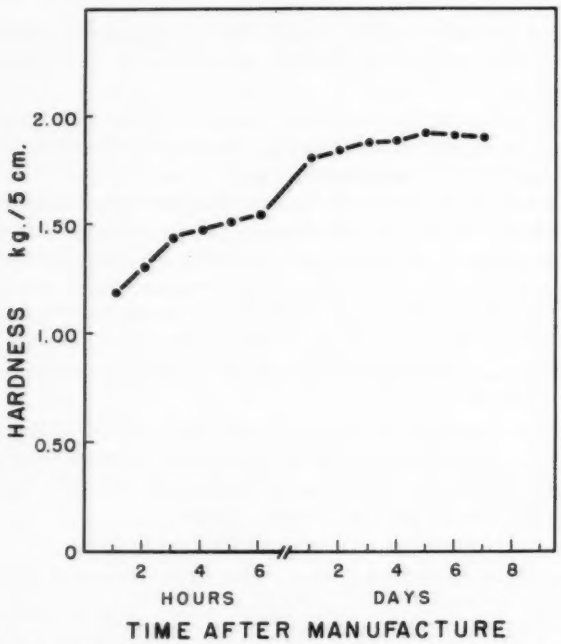

FIG. 2. Sectility hardness values during setting of continuous butter.

changes that accompanied this setting, samples of butter emerging from the texturator were immediately placed in tempered Dewar flasks. The temperature increased from 8.7 to 11.8° C. in 2 hr. (Table 4) and remained fairly constant thereafter.

TABLE 4

Temperatures of continuous and conventional butters stored in Dewar flasks

Type	Temperature ° C.						
	Initial	¼ hr.	½ hr.	1 hr.	2 hr.	3 hr.	4 hr.
Continuous	8.7	10.5	11.1	11.5	11.8	11.9	11.9
Conventional	14.9	15.0	15.0	15.0	15.0	—	—

When conventional butter was held at the temperature that prevailed when the working was completed, 12.5° C., hardness increased for eight days (Figure 3), but the temperature of the butter stored in Dewar flasks remained con-

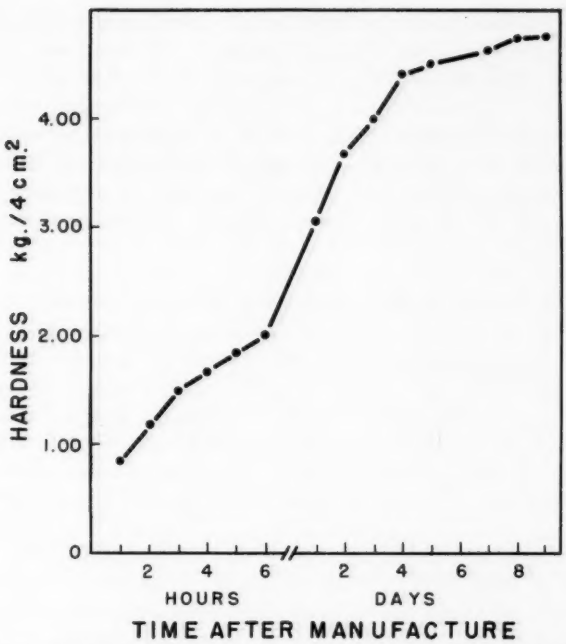

FIG. 3. Penetrometer hardness values during setting of conventional butter.

stant (Table 4). The increasing hardness of conventional butter apparently is a consequence of crystal orientation only, as the crystallization of the butterfat is essentially complete when the working of the butter is finished. In the continuously made butter, however, the hardness increase is largely caused by crystallization, and thixotropic changes are less pronounced.

When replicate samples of each type of butter were stored at 5° C., the penetrometer hardness increase was greater in the conventional butter (Table 5).

TABLE 5

Hardness changes during setting at 5° C.

Days after manufacture	Penetrometer hardness—*(kg/4 cm.²)*	
	Continuous	Conventional
1	3.90	1.76
3	3.96	2.00
7	4.12	2.34

Storage of the butters at −20° C. completely prevented setting, and no measurable hardening occurred. On subsequent removal to 5° C. storage, hardness increased to the same level as in the butters stored at 5° C. immediately following manufacture.

In a further study of the effect of storage temperature, replicate samples

of each butter type were accorded the storage treatments as set forth in Table 6, and penetrometer hardness was measured weekly. To minimize changes related to setting, all samples were stored for the first week at 5° C. Freezing temperature again interrupted hardness increase in each type of butter, but setting was resumed after the butters were removed to 5° C. storage. One week of storage of either butter at 22.5° C. had a remarkable and permanent softening effect. In investigating this phenomenon further, an equivalent softening effect required 2 wk. at 20° C., in place of 1 wk. at 22.5° C., whereas 17.5° C. was ineffective.

TABLE 6

Influence of storage temperature on butter hardness

Butter type	Treatment [a]	Penetrometer hardness—*(Kg/4 cm.²)*, after				
		1 wk.	2 wk.	3 wk.	4 wk.	5 wk.
Continuous	A	2.76	2.84	2.84	2.84	2.88
	B	2.76	2.74	2.74	2.85	2.89
	C	2.76	1.20	1.25	1.18	1.20
Conventional	A	1.88	2.10	2.12	2.16	2.16
	B	1.88	1.86	1.88	2.04	2.14
	C	1.88	1.16	1.18	1.14	1.16

[a] Treatment: A—5 wk. at 5° C; B—1 wk. at 5° C., 2 wk. at −20° C., and 2 wk. at 5° C.; C—1 wk. at 5° C., 2 wk. at 22.5° C., and 2 wk. at 5° C.

DISCUSSION

A large number of methods and instruments has been used for measuring rheological properties of butter. Unfortunately, there is uniformity neither in terminology nor in interpretation of results. Some methods have been adapted to the determination of exact physical properties, e.g., viscosity *(3, 8, 11)*; others have been linked to subjective assessments *(7, 10)*. Hardness of butter is usually measured within the range of 12-17° C., and in choosing measurement temperatures the important consideration is that the range of hardness values encountered falls within the range of the particular instrument used. In this investigation, all samples could be measured by the penetrometer method at 17° C. and by the sectility method at 12° C.

The meaning of the term spreadability is rather vague, and some investigators are of the opinion that spreadability and hardness are not directly related *(10, 12)*; on the other hand, the most widely used methods are of the penetrometer and sectility types.

Hardness has been defined as "a measure of the resistance to permanent deformation or damage" *(9)*. If this definition is accepted, penetrometer, sectility, spreadability, and other methods are all fundamentally hardness measurements, although the results are not always directly comparable.

The high correlation between penetrometer and sectility methods indicates that the same hardness property was measured; whereas, in recently reported work *(5)* on the spreadability and hardness of butter, a highly significant correlation was obtained between spreadability and sectility measurements.

The hardness of butter made by the continuous method, by comparison of corresponding weekly samples, was always significantly higher than that of conventional butter, and similar seasonal changes occurred in the two types of butter. There exists considerable difference in the physical structure of the two butters (crystal structure, gas content, water dispersion), which accounts for the dissimilar hardness. It is to be noted that the penetrometer hardness difference between the two butters does not reach the magnitude of inherent seasonal variations. Dolby *(4)* estimated that up to 80% of differences in hardness of conventional butter were caused by seasonal variations in the composition of the butterfat. Considerable interest has been shown in attempts to influence rheological properties of conventional butter by variation of temperatures before and during churning. The continuous process undoubtedly represents an extreme treatment, as the fat is subjected to rapid cooling and agitation in the chilling compartment, but even this results in penetrometer hardness changes that are low in comparison to seasonal differences.

On the basis of the limited data herein presented, it would be premature to assign to gas content a major role in butter hardness, or to proportion continuous and conventional butter hardness differences between gas content and crystal state.

The extreme hardness increase in conventional butter during the first week after manufacture is attributed to orientation of the crystal particles into a rigid structure, as no indication of crystallization was found during this period. Crystallization is still proceeding in continuous butter as it emerges from the texturator and part of the hardness increase is, therefore, caused by increase in number and/or size of crystal particles.

There was no hardness increase caused by orientation of crystal particles during storage of the butter at low temperature (−20° C.). Contrary to results reported recently *(6)*, the interruption of setting by freezing was found to be not permanent. It is probable that in the aforementioned study the butter was not allowed enough time after freezing to obtain maximum hardness, as suggested by the data reported *(6)*. The influence of setting on hardness deserves further study. At high temperature (20-22.5° C.), the crystalline structure undergoes a breaking-down process and hardness is permanently lowered. It is of interest to note that, at the latter storage temperature, both types of buttter (made at the same time of year) had approximately the same hardness at the end of the experiment.

The role of the state of crystallization in hardness differences will be the subject of further communication.

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OILING-OFF PROPERTIES OF BUTTER[1]

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SUMMARY

In this study, the oiling-off properties of conventionally churned and continuously made butter[2] have been determined.

Butter made by the continuous process had a consistently greater tendency to oil-off at 25 or 28° C. than did conventional butter. Seasonal variations throughout a 12-mo. period followed the same trend for both butter types, and were considerably greater within the type than the differences between corresponding samples of the two types. Maximum and minimum oiling-off occurred in June and October-November, respectively. This corresponds with the dates of minimum and maximum hardness of the butters of this region.

The gas content did not influence oiling-off of the continuous butter, but gas content may contribute to stickiness. Oiling-off, when expressed quantitatively as weight-percentage of the original test sample, was found to be a function of the height of the test block of butter. Temperature, as well as grade and size of filter paper, also influenced the results.

The narrowness of the temperature range in which butter exhibits acceptable physical properties is of paramount concern to the butter industry. Among these properties is that of oiliness resulting from the seepage of free oil from the butter. This condition of the butterfat may manifest itself in a weak, greasy condition of conventionally churned butter, an oily appearance of butter made by continuous methods, and an oily condition of vacuum-churned and worked conventional butter, which is also reported to show droplets of free oil frequently *(11, 12)*. This important characteristic of the texture of butter has not received the attention it deserves, particularly with regard to seasonal variability, influence of butter type, storage temperature, and standardization of measuring procedure. The increasing use of continuous methods and vacuum-churning in butter-making accentuates the need for more extensive information on the physical properties of butter.

In 1924, Hackett and Crowley *(2)* placed butter on filter paper and analysed the oil absorbed at temperatures up to 20° C. Absorption was very slow at the lower temperatures. The oil absorbed at 20° C. contained a higher percentage of unsaturated and short-chain saturated fatty acids than did the oil absorbed at lower temperatures.

Mohr and Bauer *(7)* considered that Storgård's centrifugal method presents experimental difficulty and is unsatisfactory as a measure of oiling-off. They developed the filter-paper procedure into a quantitative method. Oiling-off of summer Alfa butter was reported as being 12.5-17.7%, and of summer conventional butter as being 9.5-11.6%, when measured at 25° C.

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[1] Financial assistance for this research was provided by the Canada Department of Agriculture, Ottawa, Canada.

[2] Made by a process known as Gold'n Flow, which is copyrighted by the Cherry-Burrell Corp., Chicago, Illinois.

The Mohr and Baur method has been fairly widely used. King by this method found the oiling-off of Swedish Alfa butter (no season reported) to be 11.8-17.5% *(5)*. The Danish range of 5.9-15.7% at 20° C. for conventional summer butter, tempered for packaging directly from the churn *(10)*, is broader than that of Mohr and Baur. The high values at this temperature are not surprising, in view of the fact that the butter is discharged from the churn at an elevated temperature. The major modification of the Mohr and Baur method used by Dolby *(1)* on New Zealand butter at 18° C. prevents comparative evaluation of his results.

METHOD

It is difficult to cut a perfect cube of butter with a stretched wire. For this reason, a special forming device or trier was fabricated from a stainless steel sheet. This trier was about 7 cm. long and had a 2.5 by 2.5 cm. internal cross section. When pushed into the butter at 5° C. and withdrawn, it removed a plug of butter of similar cross section. The butter plug was pushed out of the trier by a closely fitting plunger with an attached graduated stem and the plug was cut with a stretched wire at the 2.5-cm. length, unless otherwise stated.

The cube of butter was placed on a pile of ten Whatman No. 1 filter paper circles of 12.5-cm. diameter and of known weight. The papers and butter cube were weighed and stored at 25 or 28° C. for 48 hr. and the cube then removed. To facilitate removal of the butter, paper and cubes were kept at 8° C. for 10-20 min. During the storage period, the filter papers may absorb some moisture from the atmosphere of the storage. To serve as a control, a pile of ten similar filter papers without a butter cube was stored under the same conditions. After removal from storage, these and the papers with the absorbed oil were held in a dry atmosphere until the control returned to its original weight. The papers with the absorbed oil were then weighed. Oiling-off is expressed as the weight increase of the filter papers in terms of percentage of the weight of the original butter cube.

RESULTS

Continuous vs. conventional butter. For a period of 1 yr., butter samples were collected weekly from two local creameries; one manufactured butter by the continuous method and the other manufactured conventional butter. The weekly samples were tested for oiling-off tendencies and the 25° C. results are presented as monthly averages (Figure 1). There is a marked similarity in the curves for the two types of butter. The differences are within the range 2.0-3.1%, and they average 2.6%. Statistical analysis of the results from duplicate tests of 104 butter samples shows that the difference between the two types of butter is highly significant. It may be assumed that the composition of the fat in corresponding samples of the two butter types did not differ significantly *(13)*. Therefore, differing oiling-off properties were related to unlike physical structures.

Seasonal variability. Maximum and minimum oiling-off occurred in June and October-November, respectively, and the difference between maximum and minimum values is approximately 7% for each butter type (Figure 1). Vari-

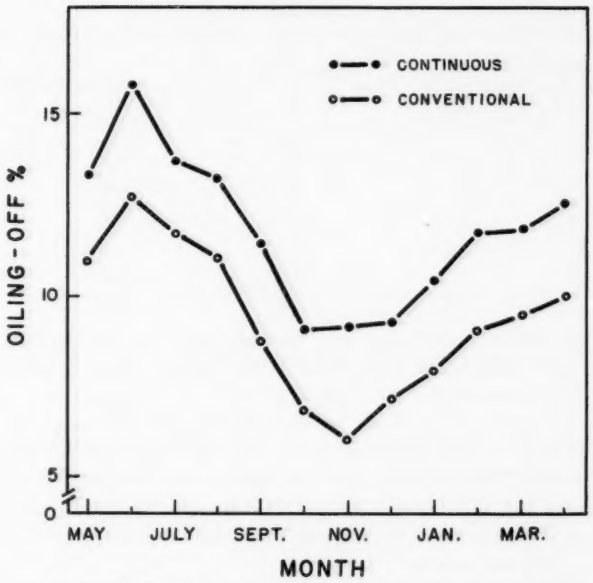

FIG. 1. Comparative oiling-off at 25° C. as influenced by season.

ability of the continuous values was within the range 9.1-15.9%, and of the conventional butters, 6.1-12.8%. Seasonal variations are considerable and of greater magnitude than differences caused by dissimilar physical structures. Seasonal variability follows closely seasonal variations in chemical composition of the butterfat. The June oiling-off maxima and October-November minima occur simultaneously with the minimum and maximum hardness of butter manufactured in this region *(6)*.

Influence of gas content. There is a major difference in the gas contents of continuous and of conventional Alberta butters. The former ordinarily contains no gas, or at least only a negligible amount, whereas the latter has a gas content of 1.5-3.5%. In order to determine the influence of gas content on oiling-off, butters of the continuous type were manufactured in the experimental continuous butter-making machine developed in this Department *(15)*. Nitrogen gas was incorporated into these butters in various concentrations (Table 1) and the oiling-off tendencies of the butters were measured. It is evident that oiling-off was not significantly influenced by even high concentrations of incorporated gas. This does not confirm the view of Mohr and Baur *(7)* that a high air content increases oiling-off. Pedersen *(9)* found that the application of vacuum during the working of butter did not influence oiling-off.

Continuously made butter characteristically lacks the stickiness of conventional butter. During these experiments the incidental, but interesting, observation was made that stickiness of the continuous type butter increased with

TABLE 1

Oiling-off of continuous type butter at 25° C. as influenced by the gas content

Butter	Gas content	Oiling-off
(No.)	*(%)*	*(%)*
1	0	17.7
1	1	18.2
1	18	18.6
2	1	19.4
2	4	20.6
2	17	20.6
3	0	17.7
3	1	17.3
3	7	17.0

increasing gas content. Differing gas contents may be the explanation of the difference in stickiness between continuous and conventional butters.

Influence of temperature. Oiling-off of both continuous and conventional butters at 28° C. was also studied, but the general picture remained unchanged. Seasonal variation followed the same pattern as that found at the lower temperature, but extended through the ranges 14.4-25.1% and 10.4-20.1% for continuous and conventional butters, respectively. There was, therefore, increased oiling-off in both types of butter and the average difference between the two types, 3.5%, was higher at this temperature.

Influence of sample size and shape. Butter plugs of varying sizes and shapes were tested to determine the influence on oiling-off. From the results (Table 2),

TABLE 2

Oiling-off at 25° C. as influenced by the size and shape of the test sample

Butter		Contact surface		Height	Oiling-off
No.	Type	Shape	Cross section		
			(cm.²)	*(cm.)*	*(%)*
1	G.F.	Square	6.25	2.5	12.7
1	G.F.	Square	6.25	5.0	7.6
1	G.F.	Rectangular	12.50	2.5	12.3
2	G.F.	Square	6.25	2.5	12.9
2	G.F.	Circular	10.54	2.5	13.0
3	Conv.	Square	6.25	2.5	11.7
3	Conv.	Square	6.25	5.0	6.9
3	Conv.	Circular	10.54	2.5	11.4

it is to be observed that oiling-off, when expressed as a percentage of the weight of the original test sample, is a function only of the height of the sample and is not related to the area in contact with the paper.

Influence of filter paper type and size. Determinations of oiling-off were made using different grades and sizes of paper, i.e., coarse (Whatman No. 4), medium (Whatman No. 1), fine (Whatman No. 5), and Whatman No. 1 with diameters of 9, 11, 12.5, and 15 cm.

From the results (Table 3), it appears that oiling-off on fine filter paper is lower than on medium or coarse paper, between which latter grades there is no

TABLE 3

Oiling-off at 25° C. as influenced by grade and size of filter paper

Filter paper		Surface area 1 circle	Oiling-off, %	
Diameter	Grade		Conventional butter	Continuous butter
(cm.)		*(cm.²)*		
12½	Fine	123	8.5	9.8
12½	Medium	123	9.6	13.0
12½	Coarse	123	9.2	12.7
9	Medium	64	9.2	11.5
11	Medium	95	9.3	12.0
12½	Medium	123	9.8	13.3
15	Medium	141	10.2	14.1

significant difference. Oiling-off also increases with increasing surface area of the filter paper.

DISCUSSION

As the physical structure of continuously made butter differs considerably from that of conventional butter, dissimilar oiling-off properties are to be expected. Indeed, the general observation has been made that the former type has a more oily appearance than the latter type of butter. The oiling-off tendency is an indication of the amount of free liquid fat in the butter, as well as of the character of the structure binding the liquid portion of the fat. Continuous butter has a much coarser crystalline structure than conventional butter *(14)*, which suggests easier drainage of the butter oil from the continuously made product. Moreover, since a considerable portion of the liquid fat in conventional butter is confined within the fat globules *(4, 8)*, a much smaller proportion can be expected to be available for drainage than with continuous butter. The difference in oiling-off between the two types of butter was fairly constant throughout the year.

Oiling-off properties may be expected to vary with the composition of the butterfat, which is subject to considerable seasonal change. According to Hilditch *(3)*, the glycerides in butterfat are of three general types, viz., (1) trisaturated, (2) monounsaturated-disaturated, and (3) diunsaturated-monosaturated. Seasonal differences in the composition of butterfat involve mainly Types 1 and 3, whereas Type 2 remains fairly constant. As Type 3 glycerides are those with the lowest melting point, it is not surprising that the seasonal variations in oiling-off were as pronounced as was demonstrated.

Since oiling-off fluctuates with such variables as temperature, height of the butter block, grade and size of filter paper, etc., the results of some of the investigations reported in the literature can not be compared closely with each other or with those reported herein. If it becomes desirable to make such comparisons, it will be necessary for investigators to use a standardized procedure.

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RELATIONSHIP BETWEEN MELTING QUALITY AND HARDNESS OF CHEDDAR CHEESE[1]

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SUMMARY

The relationship between melting quality, expressed as melting time, and cheese hardness was investigated. A relatively high correlation coefficient, +0.799, was found. Cheese hardness can be used as a means of predicting the melting quality of Cheddar cheese. The relationship of the fat content, the moisture content, and pH values to the melting quality and hardness of Cheddar cheese was investigated. These factors influenced hardness more than melting quality, but the effect, either separately or combined, was not strong enough to account for the wide variations found in melting quality or hardness.

Cheddar cheese is melted in the preparation and processing of many foods, both in industry and in the home. The term melting quality has been used to denote the ease with which cheese melts when subjected to heat. However, the term does not mean a single physical property but a combination of several, such as the time required for melting, the appearance of the melted cheese, i.e., smooth or stringy, and the amount of free fat separation. The combined effect of such factors should be considered for the specific purpose for which the cheese is melted. Thus, a cheese that has a satisfactory melting quality for one purpose may not have it for another purpose.

Study of the melting quality of cheese has been hindered by the lack of a suitable method for measuring melting quality, and the inability to predict this quality for cheese. As far as can be determined, there are no reports in the literature relating specifically to the melting properties of Cheddar cheese. Also, the available literature pertaining to the melting properties of process cheese is meager, the only information being a series of unpublished experiments *(2)* and a study by Arnott *et al. (1)* which included Cheddar cheese. Methods for evaluating the melting properties of cheese consisted either of observing standard-sized cheese samples after they had been heated over a steam bath or an electric heater for a given time *(2)*, or of measuring the height of a cheese sample before and after a heat treatment, at a constant temperature for a given time *(1)*. In part, the object of the work reported here was to attempt to correlate melting quality with certain other cheese properties, such as cheese hardness, that may be used as a means of predicting the melting quality of Cheddar cheese.

EXPERIMENTAL PROCEDURE

General. Cheddar cheese was obtained from a commercial process-cheese manufacturer. The samples were selected at random from various lots of current

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[1] The data in this paper are from a thesis presented by R. W. Weik in partial fulfillment of the requirements for the degree of M.S., University of Minnesota, Scientific Journal Series Paper No. 3856, Minnesota Agricultural Experiment Station.

and aged cheese manufactured from pasteurized milk in Minnesota, Wisconsin, Iowa, and Michigan.

A special device of stainless steel construction (Figure 1) was designed to obtain cheese samples measuring 2.25 by 2.25 by 4.50 in. Samples were taken

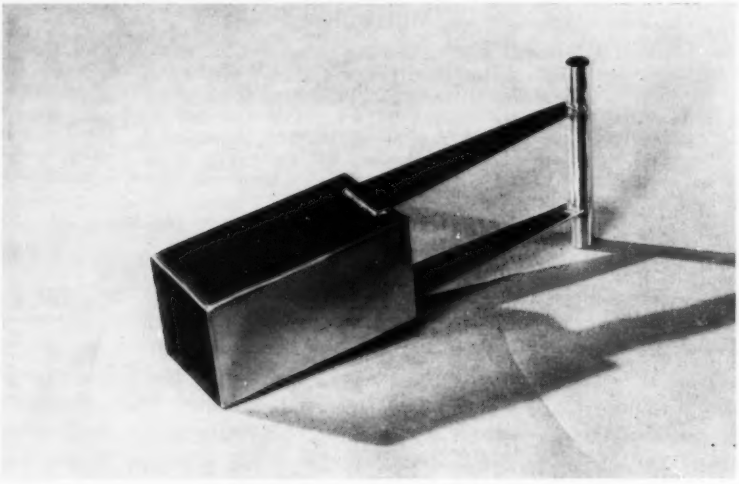

FIG. 1. Cheese-sampling device.

from each cheese in a position about one-half the distance from the center to the outer edge of the cheese.

The fat and moisture analyses were made following the method described by the Dairy Division of the Agricultural Marketing Service, USDA *(5)*. The pH values were determined as described by Arnott *et al. (1)*.

Melting quality. There are many immeasurable factors involved in the overall melting quality of cheese. Therefore, the time required for melting, an important factor, was selected as a means of evaluating melting quality. The melting time was considered an index of melting quality; a short melting time was indicative of a good melting quality, whereas a long melting time suggested a poor melting quality.

Details of the method are as follows: All cheese samples were placed in a refrigerated room, maintained at 50° F. ± 2° F., for 24 hr. prior to melting-time determinations. From the cheese sample mentioned above a standard cylinder of cheese was obtained, following the method of Arnott *et al. (1)*. The cheese cylinder was placed in an 8-in. test-tube which was closed with a cork stopper. The test-tubes containing the samples were placed in a visibility water bath[2] maintained at 80° C. ± 1° C., and the time, in seconds, required for the sample to melt was determined. The criterion for complete melting of the sample

[2] Manufactured by Blue M Electric Company, Blue Island, Illinois.

was the moment that complete loss of the original shape of the sample became evident. This method also permitted observation of differences in appearance of the cheese during melting.

Hardness. Cheese hardness was determined with the Cherry-Burrell Curd Tension Meter as modified by Irvine *(4)*. The 500-g. capacity scale supplied with the instrument was too small and was replaced with one of 1,000-g. capacity. All determinations were made in a refrigerated room maintained at 50° F. ± 2° F. Hardness was expressed as the maximum force, in grams, required to force the wire-cutter through the cheese.

RESULTS AND DISCUSSION

Melting quality. Although most of the cheese samples would melt, considerable variation was noted in the time required for melting, as well as in the appearance of the melted cheese. The majority of samples melted between 290 and 600 sec.; however, a few samples required a longer time. For example, two samples were heated for 3,180 sec. During this time no appreciable change in shape was observed.

One thousand seconds was arbitrarily selected as a practical limit for heating, since samples not melting in this length of time rarely showed any marked change in shape and did not melt completely. Samples exceeding 1,000 sec. were not included in the calculations of correlation coefficients.

Generally, the melted cheese samples requiring the least heat treatment were soft and easily stirred. The melted cheese exhibited definite plastic properties. Somewhat the reverse was observed for samples requiring melting times of 500 sec. and greater. Although melted, these samples were difficult to stir and generally quite elastic. Free fat was present, but in much smaller amounts than from the easily melted cheese.

Hardness. Theoretically, to measure cheese hardness, only the force required to break the cheese surface would have to be known. This force is the yield value or yield point. Since the yield value was difficult to determine, the hardness was taken as the maximum force required to push the wire through the cheese. Most of the cheese exhibited a hardness of less than 500 g. The range of hardness in samples was from 270 to 756 g.

The data that follow, from 89 samples of cheese, were analyzed following the method of Friedman and Foote *(3)*.

Relationship between melting quality and hardness. This relationship was considered to determine whether the just-described method of evaluating cheese hardness could be used to predict the melting quality of Cheddar cheese, expressed in terms of melting time. The relationship is shown (Figure 2). The extent of relationship is indicated by the scatter of points around the regression line and the relatively high correlation coefficient of +0.799. An inspection of the plot shows that the points are grouped fairly close around the regression line up to a hardness of about 500 g. This grouping includes approximately 65% of the samples.

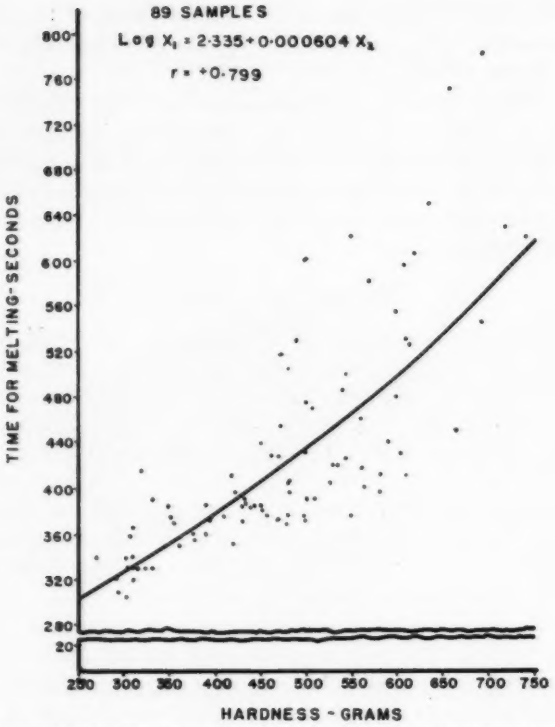

FIG. 2. Relationship of melting time to hardness of Cheddar cheese.

The coefficient of determination for this relationship was 0.638. This was interpreted to mean that the evidence presented indicated that 63.8% of the variability in melting time was associated with cheese hardness. Thus, it would appear that the relationship was high enough to use cheese hardness as a means of predicting melting quality. The degree of predictability was higher below the 500-g. level, but the trend of a longer melting time with an increase in hardness continued above the 500-g. level.

Since the coefficient of determination indicated that 63.8% of the variability in melting time was associated with cheese hardness, the remaining 36.2% of the variability must be associated with another factor, or combination of factors. On the assumption that certain constituents or characteristics of cheese would have an effect on its melting quality and hardness, the influence of the fat content, moisture content, and pH value on these characteristics was determined.

Effect of fat content, moisture content, and pH value on melting quality. The effect of these factors on melting quality was determined separately, followed by a determination of their multiple effect. The simple and partial correlation coefficients are presented (Table 1).

The effect of the fat content on melting quality, $r = +0.133$, has no statisti-

TABLE 1

Influence of various factors on the melting quality (X_1) of Cheddar cheese

Factor	Correlation coefficients	
	Simple	Partial
Fat content *(%)*—X_2	r12 = +0.133	r12.34 = a [b]
Moisture content *(%)*—X_3	r13 = −0.118	r13.24 = a
pH value—X_4	r14 = +0.234 [a]	r14.23 = +0.103

[a] Significant at the 0.05 level.

[b] a = Less than 0.001.

cal significance and is too low for any practical use in predicting the melting quality of cheese. The results are of the same magnitude as those reported by Arnott *et al. (1),* who used a different method for evaluating melting quality.

The lack of relationship between the moisture content and melting quality is shown by the correlation coefficient of −0.118, which confirms the results of Arnott *et al. (1).* An insignificant value, statistically, this value is also too low to warrant use of the moisture content as a means of predicting the melting quality.

The relationship of pH to melting quality is indicated by a correlation coefficient of +0.234. Although statistically significant, this value does not indicate a degree of relationship strong enough to use the pH value alone as a means of predicting the melting quality of Cheddar cheese.

The partial correlation coefficients were obtained, to compare the simple correlation coefficients just presented with the net effect of each one of the three factors on the melting quality, after the full effect of the two other factors was considered.

The effect of the fat content on melting quality (r 12.34) was not significantly different from zero when the influence of the moisture content and pH value was considered. A similar result was obtained for the relationship between the moisture content and melting quality (r 13.24) when the effect of the two other factors was considered. A value of +0.103 was obtained for the influence of pH on melting quality (r 14.23) when the effect of the fat and moisture content was considered. It will be remembered that the values presented for the simple correlation coefficients were small and of no practical importance regarding the predictability of melting quality. The partial correlation analysis presents no further evidence that would indicate a stronger relationship between any of the factors and melting quality. However, the values, r 14 = +0.234 and r 14.23 = +0.103, support the conclusion of Eckberg and Mykleby *(2)* that a low-pH cheese showed a tendency toward better melting quality, and a high-pH cheese a tendency toward poor melting quality.

Since a slight relationship was found between melting quality and fat content, moisture content, and pH value, the multiple effect of these three factors together on melting quality was determined. The regression equation was calculated to be:

$$\log X_1 = 1.691 + 0.0105X_2 + 0.00206X_3 + 0.0919X_4$$

where X_1 represents melting time, X_2 represents the fat content, X_3 represents

the moisture content, and X_4 represents the pH value. This equation indicates that the logarithm of the melting time and, therefore, the melting time, would increase with an increase in all factors considered. However, the degree to which variations in the melting quality were related to the combined action of the fat content, moisture content, and pH value was small, the coefficient of multiple correlation being only 0.220. This analysis indicates that although the combined influence of the pH value, fat and moisture contents influences the melting quality more than the separate effect of the fat and moisture contents, the combined influence of these three factors is not sufficient for use in predicting the melting quality of cheese.

Effect of the fat content, moisture content, and pH values on cheese hardness. The effect of these three factors on cheese hardness was considered in a manner similar to that for melting quality. The simple and partial correlation coefficients are shown (Table 2).

TABLE 2

Influence of various factors on the hardness (X_1) of Cheddar cheese

Factor	Correlation coefficients	
	Simple	Partial
Fat content *(%)*—X_2	r12 = +0.349 [b]	r12.34 = +0.181
Moisture content *(%)*—X_3	r13 = −0.347 [b]	r13.24 = a [c]
pH value—X_4	r14 = +0.245 [a]	r14.23 = a

[a] Significant at the 0.05 level.
[b] Significant at the 0.01 level.
[c] a = Less than 0.001.

The relationship of the fat content to cheese hardness is indicated by a correlation coefficient of +0.349. Although highly significant statistically, this value indicates that approximately 12% of the variation in hardness is associated with the fat content. Thus, the fat content exerts little influence on cheese hardness.

Similar results were obtained for the relationship between hardness and the moisture content. The correlation coefficient of −0.347, although highly significant, indicates that approximately 12% of the variation in hardness is associated with the moisture content.

The effect of the pH value on cheese hardness is indicated by the correlation coefficient +0.245. Despite this statistically significant value, the pH of cheese exerts little influence on cheese hardness, only 6% of the variability in hardness being associated with the pH value.

Partial correlation coefficients were calculated to provide more information concerning the effect of the pH value and fat and moisture contents on cheese hardness. A value of +0.181 was obtained for the influence of the fat content on hardness (r 12.34) when the effect of the moisture content and pH value was considered. When the single influence of the fat content on hardness was considered, the correlation coefficient was +0.349. The apparent effect of the fat content is then markedly reduced (r 12.34 = +0.181) when the influence of the moisture content and pH value is considered.

The influence of the moisture contents on hardness (r 13.24) was not significantly different from zero, when the effect of the fat content and pH value was considered. A similar result was obtained for the influence of the pH value on hardness (r 14.23) when the effect of the fat and moisture contents was considered.

This analysis presents no further evidence that would indicate a stronger relationship between any of the factors and cheese hardness. However, since the correlation coefficients were either highly significant, or nearly so, and the majority of cheese exhibited a hardness of less than 500 g., the multiple effect of these three factors on cheese hardness was determined. The regression equation was calculated to be:

$$X_1 = 207.774 + 27.373X_2 - 27.328X_3 + 60.121X_4$$

where X_1 represents cheese hardness, X_2 represents the fat content, X_3 represents the moisture content, and X_4 represents the pH values. This equation indicates that hardness increased with an increase in fat content and pH value and with a decrease in moisture content. The coefficient of multiple correlation was found to be 0.409. This value, highly significant statistically, indicates that approximately 16% of the variations in cheese hardness was associated with the combined influence of the fat and moisture contents and pH values.

Although the pH values and fat and moisture contents have been shown to influence cheese hardness more than they do melting quality, the effect of these three factors, separately or combined, on cheese hardness is not strong enough to account for the wide variations found in cheese hardness.

There are many factors not included here that undoubtedly influence both melting quality and cheese hardness. Although not a part of this study, variations in cheese structure may have a great influence on both hardness and melting characteristics. This may be one of the unexplained variables mentioned in relation to melting quality and hardness.

ACKNOWLEDGMENTS

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FACTORS AFFECTING TERRAMYCIN ACTIVITY IN MILK, BROTH, BUFFER, AND WATER[1]

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SUMMARY

Studies were conducted to determine the effect of heat, storage, total solids content, bacterial metabolism, and various gases upon the degree of terramycin inactivation in milk, broth, buffer, and water. Terramycin activity was determined by a modified disk assay technique and the acid inhibition method. The rate of heat inactivation of the antibiotic at 143 and 160° F. for 50 min. followed the first-order reaction in milk and buffer but not in water. When autoclaved at 15 lb., the antibiotic was inactivated completely within 5 min. in all three media. The *Z* values for complete thermal inactivation curves for terramycin, aureomycin, streptomycin, and penicillin in milk were 48, 51.5, 36, and 49, respectively. Terramycin was more heat-susceptible than the three other antibiotics. Heat inactivation of terramycin in reconstituted milk of different solids concentrations was inversely related to the solids content. During storage terramycin lost its potency at a faster rate in the unheated than in the heated system. This may have been owing to natural microorganisms and their enzymes in the unheated media, because it was observed that the loss of terramycin potency in a broth inoculated with a raw milk bacterial suspension was directly related to bacterial multiplication. When sterile milk containing terramycin was exposed constantly to nitrogen, oxygen, or air, the antibiotic lost its potency, the greatest loss occurring with oxygen.

Terramycin (oxytetracycline), an analogue of the tetracycline antibiotics, has been shown to possess a marked effect against various microorganisms, including gram-positive, gram-negative, aerobic, and anaerobic bacteria and many viruses *(5, 6)*. When used as a therapeutic agent, it has been shown also to possess a low degree of toxicity *(4)*. It is, therefore, used singly or in combination with other antibiotics, for the treatment of various diseases. Cromley and Hagely *(3)* and Simon and Schmidt *(15)* have reported terramycin to be therapeutically effective against staphylococcic and streptococcic mastitis. Schipper and Petersen *(10)* have shown that following intravenous and intramammary administration terramycin appears in milk, blood, and urine.

Regna and Solomons *(9)* have reported that terramycin compares favorably with the more stable antibiotics now in common use. Previous antibiotic studies conducted at this laboratory have shown that plant practices like heat treatment and storage inactivate penicillin *(13)*, streptomycin *(12)*, and aureomycin *(11)*. Information relative to the effect of heat, storage, milk solids, bacterial metabolism, and various gases upon the degree of terramycin inactivation has been obtained and is presented.

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METHODS

Throughout this study, the terramycin activity was determined by the disk assay method and the acid inhibition method. In the disk assay method, an enriched agar was used as the assay medium and *Bacillus cereus* as the test organism. The enriched agar consisted of the following gram-portions per 1,000 ml: yeast extract, 5, lactose, 5, dipotassium phosphate, 5, neopeptone, 2.5, peptonized milk, 2.5, and agar, 15. The medium was adjusted to pH 6.8 before sterilization. The principle, the details of the disk assay method, and the technique of heating and storage have been described earlier *(11, 13)*. In all the phases of this study the antibiotic was added to milk, buffer, water, or broth in concentrations ranging 0.3 and 3.2 γ per millilter.

RESULTS AND DISCUSSION

Effect of various heat treatments on terramycin. The initial phase of this study was to determine the effect of various heat treatments upon terramycin in milk, 0.1 *M* potassium phosphate buffer (pH 4.7), and distilled water. Terramycin was added to the milk, phosphate buffer, and water at the rate of 0.84 to 1.0 γ per milliliter and heated at 143 and 160° F. The rates of inactivation of the antibiotic were determined following 10, 20, 30, 40, and 50 min. of heating. The data obtained are recorded (Figure 1). The following differential equation proposed by Prutton and Maron *(8)* was used in order to determine whether or not the rate of terramycin heat inactivation against time was of the first order:

$$\text{Log } (a-x) = \frac{-k_1}{2.303} t + \log a$$

Where a = initial concentration, x = loss, t = time, and k is a constant.

In Figure 1, the logarithm of the per cent of residual terramycin has been plotted on the ordinate and the time in minutes on the abscissa. The loss of the antibiotic was found to be related directly to the heat treatment. The rate of terramycin inactivation owing to heat was faster in water than in buffer or milk. In general, these results are in close agreement with those of Bohonos *et al. (2)*, who have reported that during heating, aureomycin, terramycin, and tetracycline are less labile at a lower pH than under neutral or alkaline conditions.

It was observed that the rate of heat inactivation of terramycin at 143 and 160° F. for 50 min. in milk and buffer followed the first-order reaction, because a straight-line curve was obtained when the logarithm of the concentration of the residual antibiotic was plotted against time. In water, the rate of inactivation followed the first-order reaction at 143° F. When heated at 160° F., the inactivation rate of terramycin followed the first-order reaction up to the first 30 min. of heating only, following which the inactivation rate was progressively faster and resulted in a complete loss of the antibiotic activity in 50 min.

Studies were also conducted to determine the rate of terramycin inactivation when heated at 15 lb. (approximately 250° F.) in an autoclave. An empty autoclave was heated with steam to remove air, quickly opened, the samples inserted,

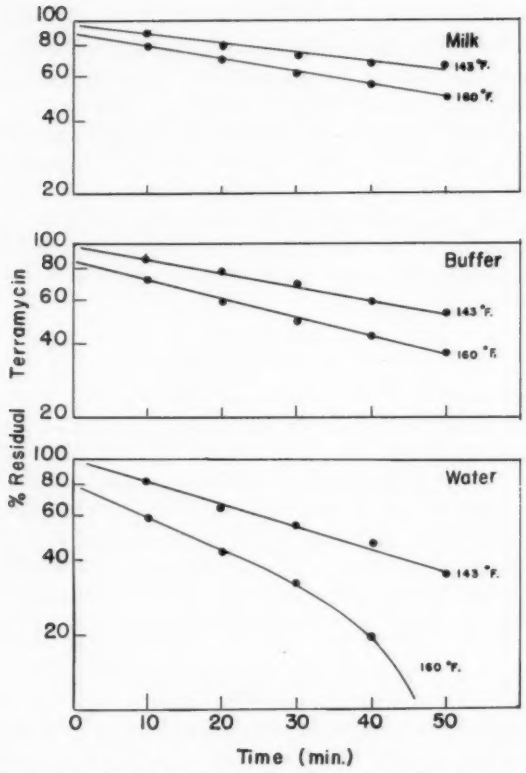

FIG. 1. Thermal inactivation of terramycin in milk, buffer, and water at 143 and 160° F.

and pressure increased quickly. As soon as the pressure reached 15 lb., the time was recorded. At the end of the desired period the pressure was reduced rapidly and the samples were withdrawn and cooled in an ice-bath. Milk, buffer, and water samples, inoculated with known amounts of terramycin, were subjected to the above treatment for 5, 10, and 15 min. and assayed for the antibiotic activity. Separate lots of the test samples were used for each time interval. It was observed that under the conditions of the above experimental procedure the antibiotic lost its potency completely within 5 min. in all three media.

Complete heat inactivation of terramycin in milk. The next phase of this study was the determination of the time-temperature relationships necessary for the complete inactivation of small concentrations of terramycin in milk. Fresh, raw, mixed-herd milk was obtained from the University dairy, and terramycin was added at the rate of 0.5 to 0.55 γ per milliliter and heated at various temperatures until the antibiotic potency was lost completely. The assay procedure included the disk assay method and also the starter activity method. As used in the previous studies *(11)*, the nondevelopment of a zone of inhibition by the first

method and no retardation in the acid development in the latter method were taken as criteria for the complete inactivation of the antibiotic. Average results of from three to six trials conducted at each temperature have been presented (Figure 2). The temperature of heating is plotted on the abscissa and the logarithm of time required for complete inactivation is plotted on the ordinate. To compare the heat stability of terramycin with that for other antibiotics, the thermal inactivation curves for penicillin, streptomycin, and aureomycin reported previously also have been included in the same figure.

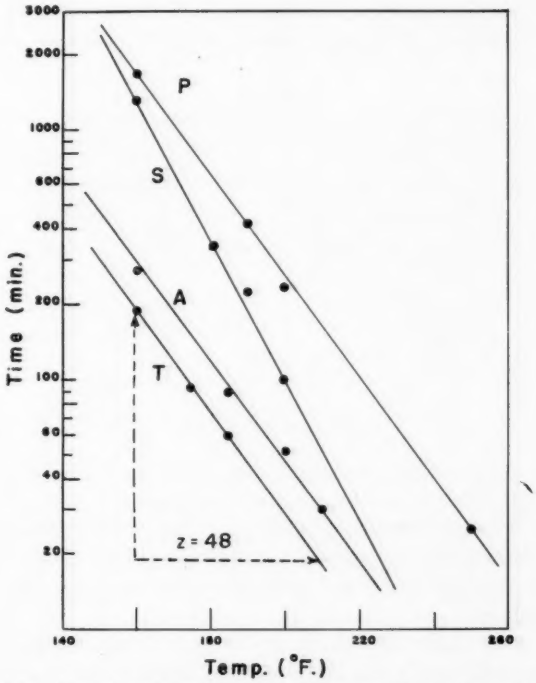

FIG. 2. Complete thermal inactivation of various antibiotics in milk. P=Penicillin, S=Streptomycin, A=Aureomycin, T=Terramycin.

Terramycin was inactivated completely in milk when heated at 160° F. for 190 min., at 175° F. for 92 min., or at 185° F. for 60 min. A linear relationship was observed between the logarithm of time and the temperature, and by using the formula proposed by Ball *(1)* the *Z* value for the thermal inactivation curve for terramycin was calculated to be 48.

The data revealed that terramycin was more susceptible to heat than were the three other antibiotics. It was observed that when heating at 160° F., 190 min. were required to inactivate terramycin, compared to 280 for aureomycin, 1,320 for streptomycin, and 1,705 for penicillin. Compared to the values of 51.5, 36,

and 49 for the thermal inactivation curves for aureomycin, streptomycin, and penicillin, respectively, the *Z* value for terramycin was 48.

Effect of milk solids upon heat inactivation of terramycin. To determine whether or not the solids content of milk was the factor responsible for the antibiotics being more heat-labile in water and buffer than in milk, a study was conducted to determine the rate of heat inactivation of terramycin in milk of various solids concentrations. Using skimmilk powder, reconstituted milk was prepared containing 10 and 25% total solids. Terramycin was added to the milk at the rate of 0.71 and 1.06 γ per milliliter, and loss of antibiotic potency was determined following heating at 160 and 185° F. for 30 min. The data obtained are presented (Table 1). Upon heating at 160° F. for 30 min., 26% of the anti-

TABLE 1

Effect of heat on terramycin in milk containing 10 and 25% total solids

	10% T.S. Milk				25% T.S. Milk			
	Heated at 160° F. for 30 min.		Heated at 185° F. for 30 min.		Heated at 160° F. for 30 min.		Heated at 185° F. for 30 min.	
Antibiotic inoculated	Antibiotic content	Loss	Antibiotic content	Loss	Antibiotic content	Loss	Antibiotic content	Loss
	(γ/ml.)	*(%)*	*(γ/ml.)*	*(%)*	*(γ/ml.)*	*(%)*	*(γ/ml.)*	*(%)*
0.71	0.58	18.3	0.30	57.7	0.59	16.9	0.28	60.5
0.71	0.57	19.7	0.31	56.3	0.60	15.5	0.30	57.7
1.06	0.72	32.1	0.30	70.7	0.90	15.1	0.47	55.7
1.06	0.70	33.9	0.34	67.9	0.88	17.0	0.46	56.6
Average loss		26.0		63.1		16.1		57.6

biotic was lost in milk with 10% solids, compared to a 16% antibiotic loss in milk containing 25% solids. A similar trend was obtained when the samples were heated at 185° F. In reconstituted milk with 10% total solids there appeared to be a direct relationship between the terramycin content of the samples and the extent of heat inactivation of the antibiotic. However, this was not true in the 25% total solids milk.

Attempts were made to determine the loss of antibiotic activity in 40% solids milk, also. However, the samples could not be assayed accurately, because the milk became too viscous upon heat treatment and could not be pipetted. Data revealed that heat inactivation of terramycin in reconstituted milk of different solids concentrations was inversely related to the solids content, indicating that milk solids perhaps exert a protective effect upon the antibiotic during heating.

Effect of storage upon terramycin in raw and heated samples. To determine the effect of storage upon terramycin in raw and heated milk, phosphate buffer, and water, the samples were inoculated with the antibiotic at the rate of 0.32 to 3.22 γ per milliliter. Using the same experimental procedure described in previous reports *(10, 11)*, the milk, buffer, and water samples were heated at 143 and 160° F. for 30 min. The raw and processed samples were then stored at from 2 to 4° C. and assayed every week for 5 wk. The per cent losses of terra-

mycin in the three media upon heating and storage are presented (Table 2). These data represent average results of from two to six trials.

Similarly to what was observed in a previous experiment (Figure 1), the heat inactivation of the antibiotic was found to be related directly to the extent of heat applied, and the rate of loss of terramycin in milk, buffer, and water was in an increasing order. There were considerable variations between the results obtained in various trials. As observed in previous studies *(11–13)*, there was no apparent relationship between the concentration of the antibiotic and the percentage loss of terramycin upon heating.

TABLE 2

Per cent loss of terramycin potency in milk, buffer, and water owing to heat treatment and storage

Heat treatment	No. of trials	Terramycin inoculated	Loss upon heating	Cumulative loss upon storage				
				1 wk.	2 wk.	3 wk.	4 wk.	5 wk.
		(γ/ml.)		*(%)*				
			Milk					
Raw	6	0.32-1.29	.....	13.9	21.8	28.2	33.0	41.9
143° F. for 30 min.	3	0.32-0.90	23.6	5.8	8.6	10.8	12.5	20.2
160° F. for 30 min.	3	0.40-1.29	35.6	1.5	6.4	.....	12.8	16.3
			Buffer					
Unheated	6	0.43-3.22	.....	18.0	21.4	25.8	28.9	33.9
143° F. for 30 min.	3	1.00-3.22	32.4	12.3	14.1	16.9	17.4	24.7
160° F. for 30 min.	2	0.43-1.28	48.4	.....	.....	15.2	21.2	22.6
			Water					
Unheated	6	0.43-3.22	.....	19.3	32.1	34.4	39.0	48.9
143° F. for 30 min.	3	1.00-3.22	41.4	4.7	10.6	12.7	19.0	25.1
160° F. for 30 min.	3	0.43-1.28	66.4	.....	13.1	.....	25.7	27.3

It was observed that during storage terramycin lost its potency at a faster rate in the unheated samples than in the heated ones. Similar results were obtained with penicillin, streptomycin, and aureomycin in previous studies. In the milk group, during storage for 5 wk., an average of 41.9% of terramycin was lost in the raw samples, compared to 20.2% in the samples that had been heated at 143° F. and to 16.3% in the samples heated at 160° F. Essentially similar trends were observed in the buffer and water samples. Under the conditions of this study there was no relationship between the terramycin content and the loss of its activity during storage. The fact that the antibiotic activity decreased during storage is in contrast to the reports of Regna and Solomons *(9)*, who observed that an aqueous solution of terramycin hydrochloride at pH 3.0–9.0 did not show detectable loss in activity during storage at 5° C. for one month.

In another study relative to the preservation of milk with antibiotics *(14)*, it was observed that during storage raw milk samples inoculated with terramycin, aureomycin, streptomycin, or penicillin developed putrefaction, proteolysis, and microbial deterioration in from ten to 14 days. In milk inoculated with terramycin or aureomycin and then pasteurized, no microbial spoilage was observed for from 3 to 4 wk. Although in the present study no attempts were made to determine bacterial numbers in the raw and heated milk, on the basis of the

study just mentioned *(14)* it may be hypothesized that during storage there was greater bacterial metabolism in raw milk than in heated milk.

Effect of bacterial metabolism on terramycin activity. Because the inactivation of terramycin during storage was faster in raw milk than in heated milk, studies were made to determine the effect of the microorganisms present in raw milk upon the antibiotic activity.

Raw milk was inoculated with terramycin and held at three temperature ranges—at 2–4, 22–26, and 35° C. Terramycin activity was determined following one, two, and three days of storage. The samples, held at from 2 to 4° C., did not show any appreciable loss of antibiotic activity. The loss of antibiotic activity could not be determined in the samples held at the higher temperatures, because the samples could not be assayed, owing to coagulation of milk. Attempts were made, therefore, to determine the effect of the microorganisms present in raw milk upon the antibiotic in enriched broth.

A raw milk bacterial suspension was prepared by inoculating raw milk in the enriched broth (composition the same as used in the enriched agar, except for agar) and incubating it at 30° C. for from 14 to 16 hr. The broth culture was transferred daily in fresh broth and was used in these trials after it had been transferred from six to seven times. Two 100-ml. lots of enriched broth containing 0.60-0.62 γ of terramycin per milliliter were inoculated with the broth suspension of raw milk bacteria at the rate of 1 and 5%, respectively. Each lot was divided into two parts. One part of each lot was incubated at 35° C. and the other part was held at 2° C. in a refrigerator. The antibiotic content and total bacterial counts were determined immediately and following one, two, and three days of incubation. The samples were stored for a maximum of three days, in contrast to 5 wk. in the immediately preceding phase of the work (Table 2), because the preliminary trials revealed that the antibiotic loss was considerably faster in the broth inoculated with a bacterial suspension than in the unheated milk, buffer, or water. Also, because the medium composition and incubation temperature influence the type and numbers of bacteria in a medium, the present phase of work was conducted not to duplicate but to investigate what might happen in raw milk.

Average results of from two to three trials are presented (Figure 3). It was observed that during holding at both the temperatures there was an increase in the bacterial count and a concomitant decrease in the antibiotic activity. As should be expected, there was greater bacterial multiplication at 35 than at 2° C. The rate and amount of the antibiotic loss were greater in the samples inoculated with 5% suspension or incubated at 35° C. than in the samples inoculated with 1% suspension or incubated at 2° C. The results clearly indicate that the loss of the antibiotic potency was directly related to the extent of bacterial metabolism. The present results are in close agreement with observations made in a previous study *(12)*, in which it was observed that in 2 wk. there occurred an appreciable loss of streptomycin activity in milk stored raw, whereas no apparent loss of the antibiotic occurred in sterilized milk. These results are not in complete agreement with the findings of Jurtshuk *et al. (7)*. They observed that zones

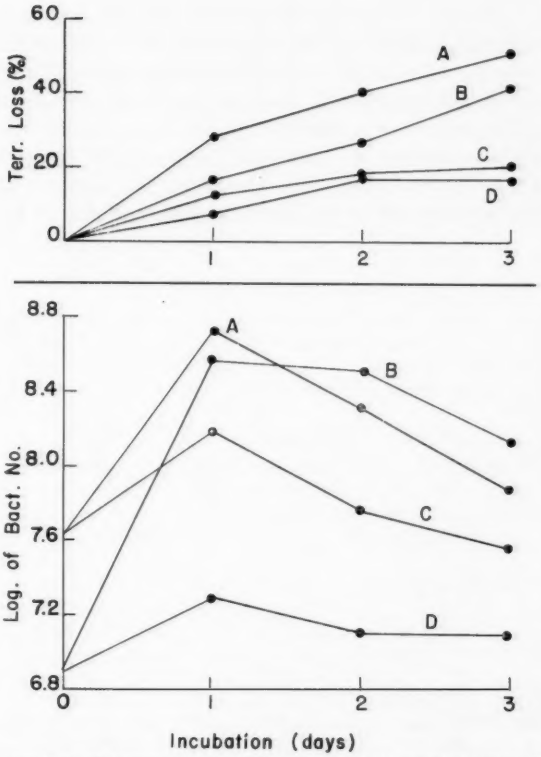

FIG. 3. Per cent loss of terramycin and rate of bacterial multiplication during incubation of broth inoculated with raw milk bacterial suspension. Inoculum: A=5%, B=1%, C=5%, D=1%, and incubation temperatures, 35, 35, 2, and 2° C., respectively.

of inhibition produced by residual aureomycin after incubation with mixed suspensions of bovine rumen bacteria were smaller than with aureomycin incubated with buffer. This was termed by them to be owing to some nonspecific type of inactivation. However, when similar work was conducted with terramycin, they found no appreciable differences between zone diameters obtained by terramycin incubated with buffer and terramycin incubated with rumen bacteria.

Effect of different gases upon terramycin activity in milk. In the search for causes and mechanisms involved in the inactivation of antibiotics, a study was conducted to determine the effect of various gases in air upon terramycin activity in milk. Twenty-five milliliter aliquots of fresh sterile milk were inoculated with from 0.96 to 1.1 γ terramycin per milliliter and dispensed in 25 $\times$ 200 mm. pyrex test-tubes with a side opening near the top of the tube. The tube was fitted with a rubber stopper with a narrow glass tubing running from the top to the bottom of the tube. Through the center glass tube, the milk was subjected to constant exposure to air, nitrogen, or oxygen by connecting the glass tubing to the

gas supply and constantly bubbling the gas through the milk system. The flow of the gas was controlled so as to produce about 50 to 60 bubbles per minute. All of these trials were conducted at room temperature (22 to 27° C.). An extra tube not containing the center tube and the side opening, and through which no gas was bubbled, served as a control. At the end of 4, 24, 36, and 48 hr. of exposure, aliquots of milk were withdrawn and assayed for antibiotic activity.

Average results of three trials are presented (Figure 4), which show the per cent loss of the antibiotic in milk upon constant exposure to various gases.

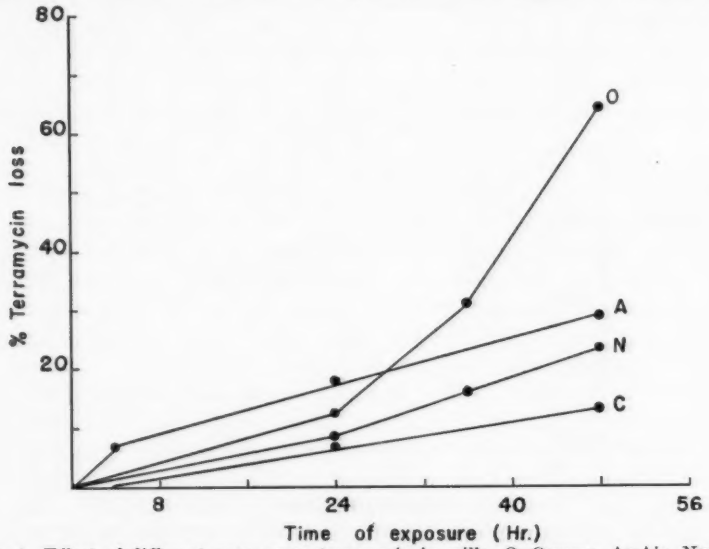

FIG. 4. Effect of different gases upon terramycin in milk. O=Oxygen, A=Air, N=Nitrogen, C=Control.

It was observed that the control samples not exposed to any gas lost about 13% of their antibiotic content in 48 hr. When milk containing terramycin was exposed to nitrogen, oxygen, or air, the samples lost part of their antibiotic activity and the loss was directly related to the time of exposure. Over a long period of exposure, oxygen was found to be more detrimental than air or nitrogen. At the end of 24 hr. of exposure to nitrogen, air, or oxygen, there resulted a loss of 8.4, 17.7, and 12.5%, respectively. Upon additional exposure of 24 hr., or at the end of a total exposure of 48 hr., 23.3% terramycin was lost in milk when exposed to nitrogen, 28.9% when exposed to air, and 64.5% when exposed to oxygen. The greater inactivation of the antibiotic when exposed to oxygen might be owing to oxidation of the antibiotic. The control milk samples in rubber-stoppered tubes, through which no gas was bubbled, also lost about 13% of their antibiotic content. These results suggest that the loss of antibiotic activity during storage might have been owing partly to the air present in the empty part of the tube. These observations may have significance to the dairy industry, because milk is often transported in cans or bulk tanks with air space.

CONCLUSIONS

Terramycin was found to be more heat-labile than penicillin, streptomycin, and aureomycin. The rate of the antibiotic loss was directly related to the extent of heat applied, and its heat-lability in milk, buffer, and water was in an increasing order. Heat inactivation of the antibiotic in reconstituted milk was inversely related to its total solids content. Storage loss of terramycin in milk might be owing partly to the presence of naturally present microorganisms and their enzymes in milk. Also, it is believed that the inactivation of the antibiotic during storage might be owing in part to oxidation.

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CONDUCTIVITY, PER CENT LACTOSE, AND FREEZING POINT OF MILK

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SUMMARY

Milk from individual cows and from groups of cows was examined for its freezing point and for factors affecting the freezing point.

Based on results obtained with 100 raw milk samples from individual cows, lactose was found to be the most important factor governing the freezing point of milk. Of the 62 samples with over 4.5% lactose, 23 (37%) had freezing points of −0.550° C. and below, and 6 (10%) had freezing points above the 3% water-tolerance level, namely, −0.5335° C.

Of the 38 samples with 4.5% lactose or less, none had a freezing point of −0.550° C. or below, but 22 (58%) had freezing points of above −0.5335° C.

The specific conductivity of milk varied inversely with the per cent lactose. Although in low-lactose mastitic milk a legal freezing point was observed, nonmastitic milk equally low in lactose showed freezing points above the legal minimum. Both prolonged storage of raw milk and pasteurization decreased the specific conductivity of milk and raised its freezing point.

Jackson and Rothera *(7)* have shown that lactose and the ionizable salts exert the greatest influence upon the osmotic pressure of milk, as measured by its freezing-point depression. They found that electric conductivity and per cent lactose showed a strict reciprocity, provided that the secretion of milk samples corresponded to the same period.

Coste and Shelbourne *(4)* observed that although there was no correlation between electric conductivity and per cent total solids, or between electric conductivity and total ash, there existed a marked correlation between electric conductivity and chlorine content of milk. These workers reached the conclusion that the osmotic pressure of milk was owing to the milk sugar and salts which it contained, and that an increase in the amount of lactose led to a decrease, not necessarily in the amount of salts but in the total number of their molecules and ions dissolved in milk. More recently, Barry and Rowland *(3)* have shown the inverse relationship between lactose and NaCl in milk.

In studying the effect of heat on milk, Magee and Harvey *(9)* observed that in fresh, raw milk about 26% of the total CaO was in diffusible form, as contrasted to only 20% in pasteurized milk and 15% in boiled milk. A significant reduction in Ca and Mg ions in pasteurized milk has also been reported by van Kreveld and van Minnen *(8)*.

The present work was undertaken to study further the ionic portion of milk, its concentration in different milks, and its influence upon the freezing point in raw and pasteurized milk.

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EXPERIMENTAL PROCEDURE

Most milk samples reported on in this study were obtained from animals of the College herd. All samples were collected during the fall and winter months, in close succession for each portion of the study, and were obtained from evening milkings only.

Milk samples obtained from complete milkings of one or more cows were collected in glass milk bottles of quart size, previously rinsed with distilled water of low conductivity and allowed to dry. All samples were cooled immediately after being drawn, and kept in ice water until analyzed, with the exception of those samples analyzed while still warm. The latter samples were kept in a water bath at 37° C. for up to 2 hr. Conductivity readings and freezing-point determinations were made within 2 hr. after collection of samples, unless stated otherwise.

The degree of ionization of milk was measured in ohms of resistance at given temperatures and is reported in (mhos/cm)$\times 10^4$ of conductivity. The apparatus used consisted of a conductivity bridge (Industrial Instrument Co., Model RC-16B) with an accompanying conductivity cell. This cell was equipped with two permanently platinized electrodes and a standardized thermometer. The cell constant was determined with aqueous KCl solution of recommended concentration at the onset of the experiments. Periodic checks were made during the study to verify the constancy of the cell.

The freezing point of milk was determined by means of an improved, modified, official Hortvet cryoscope, having a mechanical stirrer. The A.O.A.C. method was observed both in the operation of the instrument and in the standardization of the thermometer *(2)*. Periodic checks were made to verify the constancy of the thermometer.

Lactose was determined by means of a Rudolph (Routine Model 60) double-field polarimeter, having a vernier reading of 0.05°. An electric sodium lamp supplied the source of light.

Milk samples were precipitated and filtered according to A.O.A.C. directions *(2)*. A 400-mm. tube was used for obtaining readings which were then converted into per cent lactose, using the following formula:

$$\text{Per cent lactose} = \frac{\text{observed angle of rotation} \times \text{dilution factor}}{\text{angle of specific rotation} \times \text{length of tube in decimeters}},$$

wherein the dilution factor was derived by dividing the total weight of sample and of reagents used by the weight of the sample.

RESULTS

The data (Table 1) show the reciprocity of ionic concentration to lactose in normal and mastitic milk. Although the mastitic milk contained only 3.75% lactose in contrast to 4.65% in the normal milk, the freezing points were −0.543 and −0.542° C., respectively, the higher ionic concentration compensating for the lower lactose in the mastitic milk.

Average values of seven trials with Jersey and Holstein College herd milk yielded freezing points of −0.540 and −0.542° C., respectively, as shown (data Table 2). The Jersey herd milk was lower in conductivity and higher in lactose than was the Holstein herd milk. Both herds were fed identical rations and were sampled on the same days.

A statistical analysis made on the 100 samples considered in this study indicates that the increase in accuracy in estimating freezing-point depression, attained by considering conductivity at 37° C. as well as lactose content, is not

TABLE 1

Influence of mastitis upon conductivity, per cent lactose, and freezing point of milk

Cow	Clinical condition of quarter	Conductivity at 37° C.	Lactose	Freezing point
(No.)		*[(mhos/cm)× 10^4]*	*(%)*	*(− ° C.)*
1	Normal	56.3	4.65	0.542
2	Inflamed (advanced)	59.5	4.30	0.541
3	Inflamed (acute)	68.6	3.75	0.543

TABLE 2

Comparison of conductivity, per cent lactose, and freezing point in Jersey and Holstein herd milk

Trial	Conductivity at 37° C.		Lactose		Freezing point	
	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein
(No.)	*[(mhos/cm)× 10^4]*		*(%)*		*(− ° C.)*	
1	50.4	59.2	4.75	4.65	0.540	0.544
2	47.6	56.5	4.70	4.65	0.541	0.546
3	49.4	56.5	4.65	4.65	0.541	0.545
4	48.4	55.2	4.75	4.70	0.540	0.545
5	49.4	55.9	4.70	4.65	0.540	0.540
6	47.1	56.5	4.70	4.60	0.533	0.533
7	46.6	54.0	4.75	4.65	0.541	0.541
Av.	48.4	56.3	4.70	4.65	0.540	0.542

TABLE 3

Influence of decreasing per cent lactose in milk upon its conductivity and freezing point

Lactose in milk	Conductivity of the same milk at temperatures of		Difference in conductivity	Decrease in conductivity	Freezing point of milk
	37° C.	0° C.			
(%)	*[(mhos/cm)× 10^4]*		*[(mhos/cm)× 10^4]*	*(%)*	*(− ° C.)*
5.00	51.8	23.0	28.8	55.7	0.558
4.90	53.6	23.6	30.0	56.0	0.556
4.80	55.2	24.2	31.0	56.0	0.557
4.70	57.8	25.4	32.2	55.7	0.554
4.60	59.2	26.8	32.4	54.7	0.552
4.50	53.6	23.2	30.4	56.7	0.538
4.35	57.2	24.8	32.4	56.6	0.523
4.15	60.6	26.0	34.6	57.6	0.523
4.05	61.2	25.8	35.4	57.8	0.524
3.95	63.6	27.6	36.0	56.6	0.513
3.80	66.8	28.4	38.4	57.5	0.522
3.70	65.2	27.4	37.8	58.0	0.527

worth while, because the correlation between lactose content and freezing-point depression was 0.729, and including conductivity in a multiple correlation system yielded a multiple correlation of only 0.7292. The data (Table 3, Columns 1 and 6), obtained from a representative series of milk samples arranged in order of decreasing lactose content, demonstrates the influence of per cent lactose upon the freezing point of milk.

Of the 38 samples with 4.5% lactose or less, none had a freezing point of −0.550° C. or below, and only 16 samples met the 3% added water tolerance levels of −0.5335° C.; whereas, of the 62 samples containing above 4.5% lactose, 23 samples had freezing points of −0.550° C. or below, and only six samples fell below the 3% added water tolerance level.

Prolonged holding of raw milk at 0° C. up to 48 hr. resulted in slight decreases in conductivity and in a rise in the freezing points of herd milk (as shown

TABLE 4

Influence of prolonged holding of herd milk at 0° C. upon its conductivity and freezing point

Trial	Conductivity of milk after holding at °C. for: 1 hr.	48 hr.	Difference in	Freezing point of milk after holding at 0° C. for: 1 hr.	48 hr.	Difference
(No.)	*[(mhos/cm)× 10⁴]*		*[(mhos/cm)× 10⁴]*	*(− ° C.)*		*(°C.)*
			Jersey milk			
1	22.1	21.7	−0.4	0.541	0.539	−0.002
2	20.7	20.2	−0.5	0.540	0.537	−0.003
3	21.6	21.6	0.0	0.540	0.538	−0.002
4	22.0	21.7	−0.3	0.533	0.531	−0.002
5	21.7	21.7	0.0	0.541	0.539	−0.002
Av.	21.62	21.38	−0.24	0.539	0.5368	−0.0022
			Holstein milk			
1	25.4	25.2	−0.2	0.545	0.545	0.0
2	24.7	24.2	−0.5	0.545	0.541	−0.004
3	24.7	24.7	0.0	0.540	0.540	0.0
4	25.2	25.2	0.0	0.533	0.531	−0.002
5	24.5	24.2	−0.3	0.541	0.540	−0.001
Av.	24.9	24.7	−0.2	0.5408	0.5394	−0.0014

TABLE 5

Influence of pasteurization and overpasteurization upon the conductivity and freezing point of milk

Trial	Conductivity of milk at 25° C. when pasteurized for 16 sec. at: Raw	161° F.	169° F.	Difference between raw and pasteurized	Freezing point of milk when pasteurized for 16 sec. at: Raw	161° F.	169° F.	Difference between raw and pasteurized
(No.)	*[(mhos/cm)× 10⁴]*			*(169° F.–16 sec.)*		*(− ° C.)*		*(169° F.–16 sec.)*
1	45.8	45.0	44.8	− 1.0	0.533	0.528	0.526	−0.007
2	44.2	43.5	42.8	−1.4	0.535	0.528	0.524	−0.011
3	43.9	43.1	42.4	−1.5	0.535	0.527	0.525	−0.010
4	43.9	43.6	42.8	−1.1	0.534	0.529	0.527	−0.007
5	43.9	42.8	42.0	−1.9	0.535	0.531	0.529	−0.006
Av.	44.3	43.6	42.9	−1.4	0.534	0.529	0.526	−0.0082

in the data, Table 4). The changes observed were slightly greater in Jersey than in Holstein herd milk.

Data on the influence of pasteurization and overpasteurization of herd milk upon its conductivity and freezing point are shown (Table 5). Pasteurization at 161° F. for 16 sec. resulted in significant decreases in conductivity as well as in increases in freezing point. Overpasteurization at 169° F. for 16 sec. decreased the conductivity and increased the freezing point beyond the extent observed with properly pasteurized milk.

DISCUSSION

The interrelationship between conductivity and per cent lactose and its influence upon the freezing point of milk are evident from the data presented. Earlier findings *(1)*, that mastitic milk may have freezing points similar to those of normal milk, are substantiated in this study. But apparently this is not the case with low-lactose milk from nonmastitic animals. A possible explanation for this may be the difference in salt composition of the two types of milk. None of the low-lactose nonmastitic milk was salty to taste, whereas the mastitic milk was.

Both milk fat *(6)* and protein *(7)* have been reported to lower the conductivity of milk. This may explain, in part, the lower conductivity readings obtained with Jersey milk in contrast to those obtained with Holstein milk. Another factor known to affect electric conductivity in milk is its temperature. Temperature affects the extent of dissociation of molecules and also the rate of movement of ions *(5)*. Thus, Gerber *(6)* found the specific conductivity of milk to increase at the rate of one (mhos/cm) $\times 10^4$ per one degree rise in temperature between 10 and 30° C. The present study gave a calculated average increase in specific conductivity of 0.913 (mhos/cm) $\times 10^4$ with a range of from 0.78 to 1.075 (mhos/cm) $\times 10^4$ per degree C. Such values are in close agreement with Gerber's results, considering that the present study covered a temperature range of from 0 to 37° C.

Although a slight decrease in conductivity accompanied by an increase in the freezing point, after prolonged holding of raw milk at 0° C., may not be of practical significance, nevertheless it demonstrates that prolonged holding of milk may alter its freezing point. It is conceivable that fresh raw milk with a high initial freezing point, refrigerated for 48 hr. or more and overpasteurized, may have a freezing point above that permitted by law. The present trend in industry to prolong the life of milk, both in the raw state by low-temperature refrigeration, and by overpasteurization, may well lead to undesirable results from the standpoint of freezing point of milk. Although dilution of milk during processing may at times be responsible for a higher freezing point, this is not necessarily so.

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A COMPARISON OF DETERGENT TESTS FOR BUTTERFAT IN MILK WITH OFFICIAL METHODS

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SUMMARY

A collaborative comparison of the Schain and Dairy Products Section (DPS) tests with the official methods, the Babcock and Mojonnier tests, was performed by a group of experienced research workers. The volumetric tests were performed in duplicate by eight men; the Mojonnier test in quadruplicate by one man. Twelve cows, comprising four breeds with three cows of each breed, were used. Morning and evening milk was analyzed one day of the first, third, and fifth week.

The Babcock test has a closer correlation with the Mojonnier test than does either the Schain or the DPS test. The Schain test correlation is .9889, the DPS .9886, and the Babcock .9963, whereas a perfect correlation is 1.000.

The three volumetric tests averaged slightly higher than the Mojonnier: the Schain test 0.11% butterfat, the DPS 0.06%, and the Babcock 0.09%.

The standard deviation between testers was 0.15%, 0.18%, and 0.10% for the Schain, DPS, and Babcock tests, respectively. Therefore, the tester is a significant variable in these tests.

The butterfat content of the milk from these individual cows varied considerably, up and down, over the 5-wk. period. Differences of 1% fat were found in five out of the 12 cows, using the average of the morning and evening samples. This variation is far greater than that attributable to the analytical procedures or to the individual testers.

The dairy industry in the United States has relied on gravimetric analysis (Roese-Gottlieb or Mojonnier) and volumetric analysis (Babcock) for butterfat in milk for many years. Through continued research and collaborative study, these methods have been developed into highly accurate standard procedures. They are official methods of the Association of Official Agricultural Chemists and are incorporated in the laws of many states.

Alternative fat tests have been studied previously *(2, 8, 10, 11, 17, 18)*, but the development of tests that employ synthetic detergents was stimulated by the work of Schain *(15, 16)*, published in 1949 and 1950. Later work by Sager

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[1] In collaboration with R. Bassette, M. Keeney, J. F. Mattick, and H A. Newlander (present address: Meadow Gold Products Co., Washington, D. C.), Dairy Husbandry Department, University of Maryland, College Park, Maryland; F. M. Grant and R. Trimble (retired), Animal Husbandry Research Division, Agricultural Research Service, USDA, Beltsville, Maryland; E. M. Parrish, Department of Public Health, District of Columbia, Washington, D. C., and H. E. Vettel, Eastern Utilization Research and Development Division, Agricultural Research Service, USDA, Washington, D. C.

and coworkers *(12, 13, 14)* resulted in the Dairy Products Section (DPS) detergent test. Both of these tests avoid the use of strong sulfuric acid, a definite liability of the Babcock test. The DPS test requires more equipment and is more complicated to perform than the Schain test, but avoids the use of the empirical nomogram required to calculate the results of the latter test.

A survey of the published results of these detergent tests *(3-7, 12-16)*[2] revealed a need for a careful comparison of them with the official methods. The experiment reported herein was, therefore, designed and carried out cooperatively by the EURDD, Animal Husbandry Research Branch, District of Columbia Health Department, and the Dairy Husbandry Department of the University of Maryland. The contribution of the University of Maryland must be especially acknowledged for, besides providing the analytical work of the four testers, the samples of milk, and the calibration of glassware, it performed other essential parts of the experiment. The design of the experiment was developed by the Biometrical Services, ARS, in cooperation with AMS. The Biometrical Services analyzed the data.

EXPERIMENTAL DESIGN

The experiment was designed to compare the accuracy of the Schain, the Dairy Products Section (DPS), and the Babcock tests with the Mojonnier (Roese-Gottlieb) ether extraction method for determination of butterfat; and to determine the factors affecting the precision of these methods in the hands of competent analysts.

In order that an accurate, statistical comparison of these methods would be made, it was decided to test 12 cows, comprising four breeds, with three cows of each breed. The three within each breed, i.e., three Guernsey, three Holstein, three Ayrshire, and three Jersey, were chosen as far as possible to be high, medium, and low fat producers of the respective breed. Morning and evening milk of each cow being tested was analyzed for butterfat content.

As required by the design, nine testers were assigned to perform the analyses. One tester analyzed every sample for butterfat content by means of the Mojonnier method. These determinations were performed in quadruplicate by Tester A. The results of these tests were considered to be the actual per cent of butterfat in the sample being tested. All results of the other methods of butterfat determination studied were compared with these Mojonnier results.

The eight other testers, B, C, D, E, F, G, H, and I, analyzed every sample by the methods of Schain, Dairy Products Section, and Babcock butterfat tests, except for one set not analyzed because of illness and the unavoidable loss of a few samples. The tests were run in duplicate; the results were recorded as individual readings for each test performed, rather than as averages of the duplicates.

The experiment was conducted over a 6-wk. period consisting of 3 wk. of

[2] The valuable study of E. O. Herreid and collaborators (J. Assoc. Offic. Agricultural Chemists, **40**: 499. 1957.) was reported after the completion of the experimental work reported herein. The results of the two studies are in general agreement but, because of their differing purposes, can not be closely correlated.

testing and 3 wk. of assembling the data. The 1st, 3rd, and 5th wk. were the actual testing weeks. The experimental program for testing was as follows: On the first day of the first testing week (Tuesday, October 3, 1955), the Guernsey breed was tested, i. e., the morning *(m)* and evening *(e)* milks of the high, medium, and low test cows of the Guernsey breed were analyzed by Tester A, who used the Mojonnier method of testing (see Methods of Analysis). The other eight testers, B, C, D, E, F, G, H, and I, tested aliquots by the Schain, DPS, and Babcock tests. On the second day of the first testing week, the same procedure was carried out with the Holstein breed; on the third day with the Ayrshire breed, and one the fourth day of testing the Jersey breed was tested in a similar manner. In the 2nd and 3rd wk. of testing the same plan was followed.

PROCEDURE FOLLOWED IN CARRYING OUT THE STUDY

A. *Equipment and materials.* The equipment was carefully selected and checked, since emphasis was placed on accuracy. The glassware was standardized by the Dairy Inspection Service of the State of Maryland. All glassware which did not meet the requirements of the State was discarded. It was also ascertained that the speed of weighing might also be a factor in the accuracy of the tests to be performed. Therefore, Mojonnier dishes were purchased which were tared to within 3 mg. of each other. Thus, only a limited amount of weight-adjusting was necessary for each aliquot of each sample, which was accomplished on a rapid indicating balance.

The chemicals and reagents used in the testing study were as follows: The sulfuric acid used in making the fat determinations by the Babcock method had a specific gravity of 1.84; therefore, each tester had to standardize his own acid to a specific gravity of 1.82-1.83, which is the specific gravity of the sulfuric acid designated Babcock acid. The Schain reagent was obtained in one lot of eight individual gallons from Dr. Philip Schain of Staten Island, N. Y. The sodium tetraphosphate (Quadrafos)[3] was obtained from the Rumford Chemical Company, Rumford, Rhode Island, and the methyl alcohol (99.6%) from Mojonnier Brothers,[3] Chicago, Illinois. The alcohol was standardized by the individual testers to 50%. Triton X-100[3] and Quadrafos were obtained in a large container and portions were distributed to the testers, because these chemicals were not available in small lots. All other chemicals were purchased in individual containers. Each tester was supplied with the necessary equipment (except the centrifuge and water baths) and chemicals to carry out the tests. This was done for the purpose of keeping conditions as similar as possible, to minimize sources of error.

B. *Methods of analysis.* A working handbook was prepared for each tester, that contained the method and step-by-step procedure for each test. The directions for the Babcock test were taken directly from official methods *(1)*. The Mojonnier test was run according to directions published by the company *(9)*,

[3] The mention of commercial products and manufacturers is for the purpose of identification and does not constitute endorsement by the USDA.

except that three extractions were performed and the final extraction of the fat was made using only petroleum ether and ethyl ether. This modification made the procedure essentially equivalent to the Roese-Gottlieb method, as described on page 233 of the AOAC methods *(1)*.

Testers were instructed to read their results as accurately as possible to the second decimal place, for the Babcock and for the two other volumetric tests. Directions for the DPS test were taken from the paper of Sager *et al. (14)*. The Schain test was performed according to directions described in photostatic copies of the method, as revised February 1955, with minor inked-in changes made by Dr. Schain just prior to the initiation of this study.

C. *Manipulation of samples.* The evening milk samples were obtained from the University of Maryland dairy barns by one of the cooperating testers. The milk was obtained by machine-milking and placed in individual milk cans. It was immediately taken to the dairy manufacturing building, agitated thoroughly by pouring from one container to another three times, then 9-pt. bottles of each sample were poured. The pint aliquots were stored overnight at 40° F.

The morning milk was handled in an identical fashion, except that it was not stored but was delivered to the cooperating testers as soon as possible, usually by 8 A.M. Both sets of samples were warmed to from 105 to 110° F., mixed thoroughly in the bottles, and then tempered to the standard temperatures of analysis before pipetting the samples.

RESULTS

The data before analysis are not presented, because of their great number. Photostats of the tabulated results can be obtained by writing to the senior author.

Averages of the volumetric analyses by the eight testers, for each sample plotted against the Mojonnier data, are shown (Figure 1). Certain features of the data, such as the fact that most of the samples had fat contents between 3 and 6%, and that the standard deviation for the DPS and Schain tests is greater than for the Babcock test, are apparent by inspection of this figure.

The experimental design called for the collection and analysis of 72 samples of milk; two from each of the 12 cows in each of the three testing weeks. However, three samples were not analyzed, because they were either lost or churned before reaching the tester. In addition, some of the testers were unable to obtain determinations on several of the remaining 69 samples. The loss of determinations, as reported by the eight testers (B through I), with the Schain and DPS tests, was almost five times and three times as great, respectively, as with the Babcock test. Loss of determinations with the Babcock test was negligible.

The four determinations with the Mojonnier (by Tester A) on each of the 69 samples were averaged and compared with the averages of the duplicates for each of the other tests, separately, for each of the eight testers. In addition, the 69 samples were divided into a high and low group, based on the fat content as determined by the Mojonnier method. The high group consisted of 35 samples, with the average Mojonnier reading of the lowest sample being 4.234% fat and

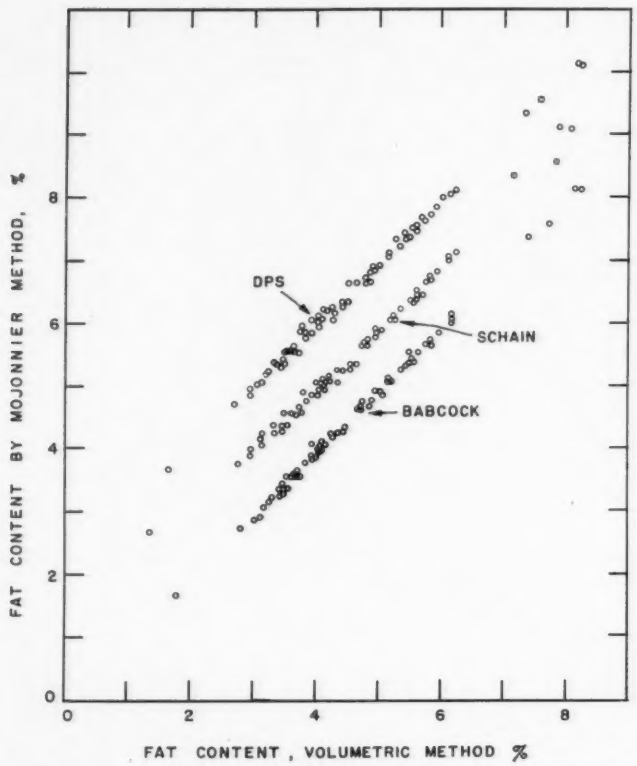

FIG. 1. Average of the volumetric analyses for the eight testers *versus* average of Mojonnier run in quadruplicate by one tester. The ordinate is raised by 1% for the Schain test and by 2% for the DPS test, that is, all points are arbitrarily raised by these amounts to separate the data for the three sets of results.

of the highest sample, 8.118% fat. The low group consisted of the 34 other samples, with the low sample containing 1.658% fat and the high sample, 4.218% fat. The reason for placing the samples into two groups was to determine if the linear regression of the Mojonnier reading on the Schain, DPS, or Babcock tests differed at different fat levels, which would be good evidence of the existence of curvilinearity. A summary of the linear regression and simple product-moment correlation coefficients is given (Table 1).

The difference between the regression in the low group and regression in the high group is 0.175, 0.046, and 0.022 for the Schain, DPS, and Babcock tests, respectively. These results suggest that the true relation between the Mojonnier and Schain tests can best be described with a curved, rather than a straight, line. The relation between the Mojonnier and DPS tests appears to be only slightly curvilinear; whereas, the Mojonnier and Babcock tests are essentially linear in

TABLE 1

Intratester correlations and regressions of the Mojonnier Test with the Schain, DPS, and Babcock Tests

Groups based on fat content	Schain	DPS	Babcock
		Regressions	
Low	0.851	0.916	1.011
High	1.026	0.962	0.989
All samples	0.964	0.961	0.991
		Correlations	
Low	0.9647	0.9533	0.9815
High	0.9798	0.9774	0.9936
All samples	0.9889	0.9886	0.9963

their relation to each other. Additional samples are needed, especially in the upper and lower fat levels, in order to establish these relations accurately.

It should also be noted (Table 1) that the Babcock test is more closely correlated with the Mojonnier reading than with the two other tests. Although these differences are not great, they are responsible for differences in the standard errors of estimate, which are significant statistically (as can be seen in Table 2). The linear regression and simple product-moment correlation coefficients of the Mojonnier test with the Schain, DPS, and Babcock tests were calculated separately for each of the eight testers. These did not vary materially from one tester to another. However, the average difference of the Mojonnier test and each of the other tests varied widely between testers. These differences and the standard errors of estimate are given (Table 2) for all samples and for each tester separately. The standard deviation between testers is given at the bottom of the table for each test.

Only one tester (C) had a larger standard error of estimate for the Babcock test than for either of the two detergent tests. The standard errors of estimate for all 69 samples on an intratester basis were 0.17, 0.18, and 0.10 for the Schain, DPS, and Babcock tests, respectively.

The variability between testers within samples, as measured by the standard deviations (bottom of Table 2), originates from three possible sources. These are *(1)* differences that would exist between determinations (using the same test) on the same sample by the same tester, *(2)* real differences between testers, and *(3)* the interaction of samples and testers. The importance of these sources of variation in the bias observed between the Mojonnier test and each of the three other tests is more critically evaluated in subsequent analyses.

Duplicate determinations. The general procedure followed by most testers in the conduct of these tests was to repeat the determinations, when the first set of duplicate determinations failed to agree within the limit set in the instructions (0.10% fat).[4] The discarded determinations were not reported by the

[4] Although the directions for the Babcock test and the Schain test call for repetition of the test, where duplicates vary by more than 0.10% fat, and where those for the DPS test do also by inference, inspection of the raw data shows that this was not always done. The analysis of variability between duplicates is affected by these facts, but the validity of the major correlations is not affected significantly thereby.

TABLE 2

Average differences from the Mojonnier Test, standard errors of estimate, and standard deviations between testers for the Schain, DPS, and Babcock Tests

		Test		
		Schain	DPS	Babcock
	D[a]	0.14	0.18	0.20
Tester B	SE of E[b]	0.12	0.09	0.07
	D	0.13	0.10	0.06
Tester C	SE of E	0.15	0.14	0.16
	D	0.08	0.14	0.16
Tester D	SE of E	0.13	0.10	0.07
	D	0.12	0.04	0.08
Tester E	SE of E	0.19	0.27	0.11
	D	0.08	−0.01	0.13
Tester F	SE of E	0.15	0.23	0.12
	D	0.07	−0.03	0.11
Tester G	SE of E	0.20	0.17	0.06
	D	0.15	0.09	0.03
Tester H	SE of E	0.13	0.19	0.08
	D	0.12	−0.05	−0.07
Tester I	SE of E	0.21	0.14	0.12
	D	0.11	0.06	0.09
All:	SE of E	0.17	0.18	0.10
	SD[c]	0.15	0.19	0.12

[a, b, c] D refers to differences from the Mojonnier, SE of E to standard errors of estimate, and SD to standard deviations between testers.

tester. The standard deviation between duplicate determinations was calculated for each tester and for the Schain, DPS, and Babcock tests separately. These standard deviations are shown (Table 3), along with the number of samples that were reported with duplicate determinations in each case.

The variability between duplicate determinations varied between testers. On the average, the greatest difference occurred between duplicate determinations that were reported for the DPS test (standard deviation =0.073), intermediate for the Schain test (standard deviation =0.052), and smallest for the Babcock test (standard deviation =0.046).

Variability among daily means. Because of missing values in some cases, it was necessary to average over the morning and evening samples from the same cow to simplify the analyses of variance. Data collected by the last tester (I) were omitted from these analyses, because he made no determinations on 12

TABLE 3

Standard deviations between duplicate determinations and number of samples in each case

	Standard deviations			No. samples		
		Test			Test	
	Schain	DPS	Babcock	Schain	DPS	Babcock
Tester B	0.035	0.044	0.027	67	69	69
Tester C	0.035	0.053	0.042	67	68	68
Tester D	0.050	0.040	0.025	65	68	68
Tester E	0.062	0.134	0.032	67	68	68
Tester F	0.044	0.041	0.090	57	57	68
Tester G	0.033	0.036	0.034	61	67	68
Tester H	0.077	0.106	0.024	67	68	68
Tester I	0.062	0.060	0.055	58	58	58
Intratester	0.052	0.073	0.046	509	523	535

samples. Both a morning and an evening sample were collected and tested for each cow, except in four instances, where one of these two samples either was not collected or had no readings by most testers. The table of daily means, set up from the original data, was a tester × test × cow × week table. The corresponding daily averages of the Mojonnier test were then subtracted from each of these values. This gave a total of 756 mean differences (biases) from the Mojonnier. These biases were analyzed by the analysis of variance for each breed and for each test separately. The mean squares of the analyses of variance for each test are given (Table 4) and the separate analyses of variance from each of the four breeds are shown (Table 5).

It should be noted (Table 4) that the error variance (M × W × C : B) is three times and five times larger, for the Schain test and DPS test, respectively,

TABLE 4

Analysis of variance of biases for each test, mean squares only[a]

	d.f.	Test		
		Schain	DPS	Babcock
Total	251	0.0368	0.0393	0.0172
Breeds	3	0.0452	0.1760	0.0174
Men (M)	6	0.0376	0.2019	0.1352[b]
Weeks (W)	2	0.1621	0.0356	0.0540
B × M	18	0.0204	0.0392	0.0074
B × W	6	0.1309	0.0232	0.0621
M × W	12	0.0559[b]	0.0798[b]	0.0246[b]
B × M × W	36	0.0246[b]	0.0450[b]	0.0078[b]
Cows (C:B)	8	0.2840	0.1303	0.1042
M × C:B	48	0.0085	0.0147	0.0024
W × C:B	16	0.1382[b]	0.0851[b]	0.0630[b]
M × W × C:B	96	0.0098	0.0160	0.0031

[a] Appropriate error terms were determined by assuming that all effects except breeds were random.

[b] Significant at the 0.01 level.

TABLE 5

Analyses of variance of biases for each breed, mean squares only[a]

	d.f.	Breed			
		Guernseys	Holsteins	Ayrshires	Jerseys
Total	188	0.0646	0.0323	0.0118	0.0147
Men (M)	6	0.0604	0.0467	0.0396	0.0809
Tests (T)	2	0.0681	0.1367	0.0349	0.0050
Weeks (W)	2	0.5938	0.0082	0.0505	0.0386
Cows (C)	2	1.5131	0.1107	0.0682	0.0281
M × T	12	0.0774[b]	0.0631	0.0077	0.0260
M × W	12	0.0605[c]	0.0363[b]	0.0171	0.0308[c]
M × C	12	0.0137	0.0097	0.0040	0.0048
T × W	4	0.0474	0.0076	0.0211	0.0284
T × C	4	0.0579	0.0880[b]	0.0077	0.0232
W × C	4	0.9208[c]	0.0343[b]	0.0848[c]	0.0139
M × T × W	24	0.0292[c]	0.0493[b]	0.0142[c]	0.0301[c]
M × T × C	24	0.0070	0.0218	0.0040	0.0025
M × W × C	24	0.0093	0.0122	0.0080[b]	0.0053[b]
T × W × C	8	0.0190[c]	0.0144	0.0048	0.0077[b]
M × T × W × C	48	0.0054	0.0279	0.0043	0.0028

[a] Appropriate error terms were determined by assuming that all effects except tests were random.

[b] Significant at the 0.05 level.

[c] Significant at the 0.01 level.

than is the error variance for the Babcock test. These differences in error variances are significant statistically, and clearly show that sources of variation other than those considered in this study were considerably more important in the Schain and DPS test than in the Babcock test. Perhaps this can be explained by the fact that the conditions and manipulations of the Babcock test have been more highly standardized, through long study and use, than have those of the newer tests.

The week × cow interaction within breeds (W × C : B) is highly significant for all three tests (Table 4). This means that the bias in estimating the average fat content of two samples (in most cases—morning and evening) on the same cow varied from week to week, even when the same test was used. The week × cow interaction, when averaged over all tests, but separately for each breed (Table 5), is significant for the Holstein data and highly significant for the Guernsey and Ayrshire data. Changes in the average bias from week to week for data from the same cow are shown (Table 6). This interaction is more noticeable in the Guernsey breed because of one low sample collected on Cow No. 1 the first week, which was greatly underestimated on the average by the Schain test.

Analyses of variance similar to those given in Tables 4 and 5 were calculated for the daily means without regard to the Mojonnier readings. The week × cow interaction was highly significant in all of these analyses. Therefore, because the fat content of samples from these cows varied differentially by weeks and because an association was found between the amount of bias and the fat content

TABLE 6

Subclass means of biases

Breed	Cow	Weeks 1	2	3	Test Schain	DPS	Babcock	Means
	1	−0.500	0.061	0.104	−0.141	−0.117	−0.077	−0.112
	2	0.209	0.186	0.138	0.267	0.114	0.152	0.178
	3	0.151	0.140	0.096	0.144	0.086	0.157	0.129
Guernsey		−0.047	0.129	0.113	0.090	0.028	0.077	0.065
	1	0.017	0.087	−0.010	−0.015	−0.009	0.118	0.131
	2	0.029	0.033	0.024	0.114	−0.074	0.047	0.029
	3	0.087	0.080	0.141	0.102	0.091	0.115	0.103
Holstein		0.045	0.067	0.052	0.067	0.003	0.093	0.054
	1	0.095	−0.005	0.061	0.118	0.051	0.082	0.084
	2	0.118	0.088	0.061	0.098	0.071	0.099	0.089
	3	0.190	0.151	0.088	0.178	0.135	0.116	0.143
Ayrshire		0.134	0.078	0.104	0.131	0.085	0.099	0.105
	1	0.065	0.155	0.115	0.097	0.123	0.115	0.112
	2	0.120	0.144	0.140	0.165	0.117	0.122	0.135
	3	0.094	0.123	0.060	0.046	0.115	0.116	0.092
Jersey		0.093	0.141	0.105	0.103	0.118	0.118	0.113
Means		0.056	0.104	0.093	0.098	0.059	0.097	0.084

	Tester B	C	D	E	F	G	H
Schain	0.130	0.108	0.062	0.107	0.065	0.070	0.141
DPS	0.164	0.085	0.114	0.018	−0.017	−0.044	0.091
Babcock	0.191	0.047	0.150	0.065	0.114	0.097	0.013
Week 1	0.098	0.010	0.090	0.052	0.045	0.047	0.052
Week 2	0.193	0.098	0.098	0.100	0.025	0.088	0.124
Week 3	0.194	0.133	0.139	0.038	0.092	−0.112	0.069
Means	0.162	0.080	0.109	0.063	0.054	0.041	0.082

of the sample in these data, it seems safe to conclude that a considerable amount of the week × cow interactions would be eliminated, if adjustment were made for the level of fat.

The breed × men × week (B × M × W) interaction is highly significant for all three tests (Table 4). This means that the average weekly changes in the biases from one breed to another were not evaluated in the same manner by all testers. The Babcock test was considerably less variable from tester to tester, in this respect, than were the two other tests.

The highly significant men × week (M × W) interaction for each of the three tests (Table 4) indicates that these tests were not conducted in the same manner by each tester each week. Changes that took place in the test procedures, reagents, etc., were not the same for all testers. The men × week (M × W) interaction, when averaged over all tests for each breed separately (Table 5), is significant for the Holstein data and highly significant for the Guernsey and Jersey data.

Experience in collaborative studies of this type has shown repeatedly that analytical tests are more sensitive to subjective effects than it is commonly believed. The significance of the M × W interaction indicates clearly that the individual operator is from day to day a variable in the performance of these tests, regardless of extensive efforts to standardize procedures, reagents, equipment, etc., between testers.

The greatest differences between testers occurred with the DPS test. However, these differences varied so much from week to week with the DPS test that the average differences between testers are not quite significant at the 0.05 level, when the effects of weeks are considered as random. On the other hand, the average differences between testers are highly significant in the case of the Babcock test, because of less variation in these differences from week to week.

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EARLY EMBRYOLOGY OF THE COW.
I. GASTRULA AND PRIMITIVE STREAK STAGES[1, 2]

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SUMMARY

Serial sections of 16 bovine embryos recovered from cows slaughtered at 16, 17, and 18 days after insemination were studied to establish suitable standards of normal development and to define the limits of normal variation encountered during early pregnancy. The 16-day-old blastocysts were engaged in the processes of gastrulation, and ranged in development from a hollow bilaminar sphere to an elongate chorionic vesicle with an elevated germinal disk. Their principal features were the remarkably rapid growth of the trophoblast and the differentiation of somatic and splanchnic mesoderm. Blastocysts recovered at 17 days were characterized by further growth, cephalocaudal elongation of the germinal disk, and differentiations of Hensen's node and the primitive streak establishing the longitudinal axis. In the 18th day of development, the blastocysts extended throughout the major portion of the gravid horn and the embryos displayed well-defined primitive node, primitive groove, and notochord development. Amniogenesis was initiated and, in two specimens, completed amniotic sacs were present. Two cases of probable embryonic death were encountered in which the trophoblast persisted after loss and resorption of the embryonic area. The morphogenetic processes involved in gastrulation and primitive streak formation are considered in the light of reported high embryonic death during early pregnancy.

The literature pertaining to studies of the embryology of the cow is notably sparse. Hallman *(11)* studied the development of placentae from cows from 3 to 5 mo. pregnant, with special reference to placental pathology. Hammond *(13)* studied the relations between rate of development of the uterus, fetus, fetal fluids, and fetal membranes, and included brief descriptions and illustrations of a few fetal specimens recovered at the end of each lunar month of pregnancy. Hartman *et al. (14)* and Miller *et al. (23)* described a two-celled bovine egg recovered 48 hr. after mating and an unfertilized tubal ovum 72 hr. after mating. Yapp *(31)* presented data on 31 fetuses ranging from 41 to 277 days of pregnancy, emphasizing changes in body proportions during various periods of development. Kupfer *(21)* reported on a series of specimens with estimated ages from about 3 to between 15 and 17 wk. of age.

The most valuable and precise information on the embryology of the cow is provided by Winters *et al. (30)*. Employing timed specimens, they presented data and illustrations on material ranging from one-day-old ova to the new-born calf. The scope of this undertaking made it necessary to base some observations

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upon single specimens; moreover, there are a number of omissions in the day-by-day descriptions of the embryological development.

Nichols *(25)* collected data on body weights and a number of body measurements from about 100 embryos and fetuses of Hereford cows, but precise knowledge of the stages of gestation was lacking. A study of the rate of development of the gravid uterus and its contents at various stages of pregnancy was made by Swett *et al. (27)*, using data from 115 cows ranging from 14 to 276 days of gestation.

More recently, the development of the bovine ovum up to the stage of blastocyst formation has been reported by Hamilton and Laing *(12)*. Chang *(5)* described blastocysts obtained from slaughterhouse material estimated to range from about 8 to 18 days of development. Greenstein and Foley *(9, 10)* reported briefly on the development of the bovine embryo between the late blastocyst stage and early organogenesis.

The recognition of early embryonic death as an important factor in bovine infertility *(1, 3, 4, 6, 15, 16, 28, 29)* has pointed up the need for more detailed, accurate information relative to normal embryonic development during the critical stages of germ layer formation, the development of body form, and the establishment of the organ systems. This paper is the first in a series describing the day-by-day development of the cow from the late blastocyst stage to the fully formed embryo.

MATERIAL AND METHODS

Reproductive tracts were recovered from dairy and beef cows and heifers slaughtered at the University abattoir. These cows represented culls from the University herds. Detailed records were available relative to ancestry, health, age, breeding history, and reproductive performance of all individuals. The cattle were closely watched for signs of estrus, and those that came into estrus in the morning were mated at that time and again in the afternoon; those coming into heat in the afternoon were bred that day, and again the following morning if still in estrus.

Since ovulation normally occurs from 10 to 15½ hr. postestrum *(2, 13, 24)*, in calculating the ages of the embryos in this study the day after the last breeding was considered as the first day of pregnancy and the date of slaughter as the final day.

Specimens were flushed from the uterine horns with physiological saline for observation under the dissecting microscope. They were then photographed, or fixed immediately in a modified Bouin's fluid, followed by standard dehydration, clearing, and Tissuemat infiltration. The embryos were serially sectioned at 8 or 10 microns and stained with hematoxylin-eosin or a modified Mallory triple stain.

Observations. Sixteen timed embryos were available for study. A list of the embryos and their principal characteristics is presented (Table 1).

Sixteen-day embryos. Six embryos were recovered from animals slaughtered 16 days after breeding. The gross appearance of the specimens varied consider-

TABLE 1

Pregnant	Animal	Blastodermic vesicle	Stage of embryonic development	Figure
(days)	*(No.)*	*(mm.)*		
	NE-20	1.5	Bilaminar hollow sphere. Embryonic disk area distinct from trophoblast.	
	NE-93[a]	6	Early elongation of blastocyst. Dense spherical disk (0.4 mm.) elevated to surface.	1, 4, 5, 6, 7
	NE-69[b]	29	Marked growth and folding of trophoblast wall. Embryonic disk unchanged.	2
16				
	NE-16	60	Slender vesicle (1.65 mm. in diameter). Disk occupies middle portion.	
	NE-132	75	Cephalocaudal elongation of disk (0.45 by 0.6 mm.). Proliferation of mesoderm.	3, 8, 9, 10, 11
	NE-22	90	Embryonic failure. Trophoblast undergoing degeneration.	
	NE-50	70	Embryonic failure. Persistent chorionic development.	
	NE-61[a]	125	Primary germ layers well-established. Extension of extraembryonic mesoderm.	
17				
	NE-148	135	Oval disk (0.4 by 0.75 mm.) with caudal primitive groove. Possible early amniogenesis.	12, 13, 14, 15
	NE-39	150	Longitudinal axis clearly evident. Undercutting of embryonic area.	16, 17
	NE-55	240	Pronounced Hensen's node and mesodermal delamination.	18, 19
	NE-49[a]	100	Thickened ectoderm, well-formed primitive streak. Disk 0.56 by 0.72 mm.	20
	NE-85	175	Distinct extraembryonic coelom. Suggestion of primitive amniotic folds.	21, 22, 23
18				
	NE-90	Length of gravid horn	Marked primitive groove. Extensive extraembryonic coelom.	24
	NE-37	225	Well-defined amniotic sac. Emergence of notochordal elements.	25
	NE-131	Invading nongravid horn	Well-developed amnion, notochordal mass, and lateral mesoderm.	26, 27

[a] Five or more services required for conception.
[b] Previous abortion.

ably, particularly with regard to the elongation of the chorionic vesicles, ranging from 1.5 to 90 mm. in length, with a mean length of 43.6 mm. The diameters of the vesicles were relatively constant at approximately 1.5 mm. The embryonic disks were similar in all specimens, being nearly spherical, with an approximate diameter of 0.4 mm.

Specimen NE-20 appeared as a minute, bilaminar, hollow sphere with only faint evidence of an embryonic disk on its surface. Unfortunately, this specimen was lost in processing. A somewhat larger blastocyst was recovered from NE-93, as illustrated (Figure 1). The embryonic disk was imperceptible in saline solution but, with the addition of fixative, became evident as an opaque spherical structure.

Figures 4 to 7 illustrate representative 8-micron sections of specimen NE-93. The germinal disk has become completely elevated to the surface, with only an occasional vestige of overlying trophoblast or Rauber's membrane (Figure 6) remaining as detached cells. The endoderm has separated from the germinal

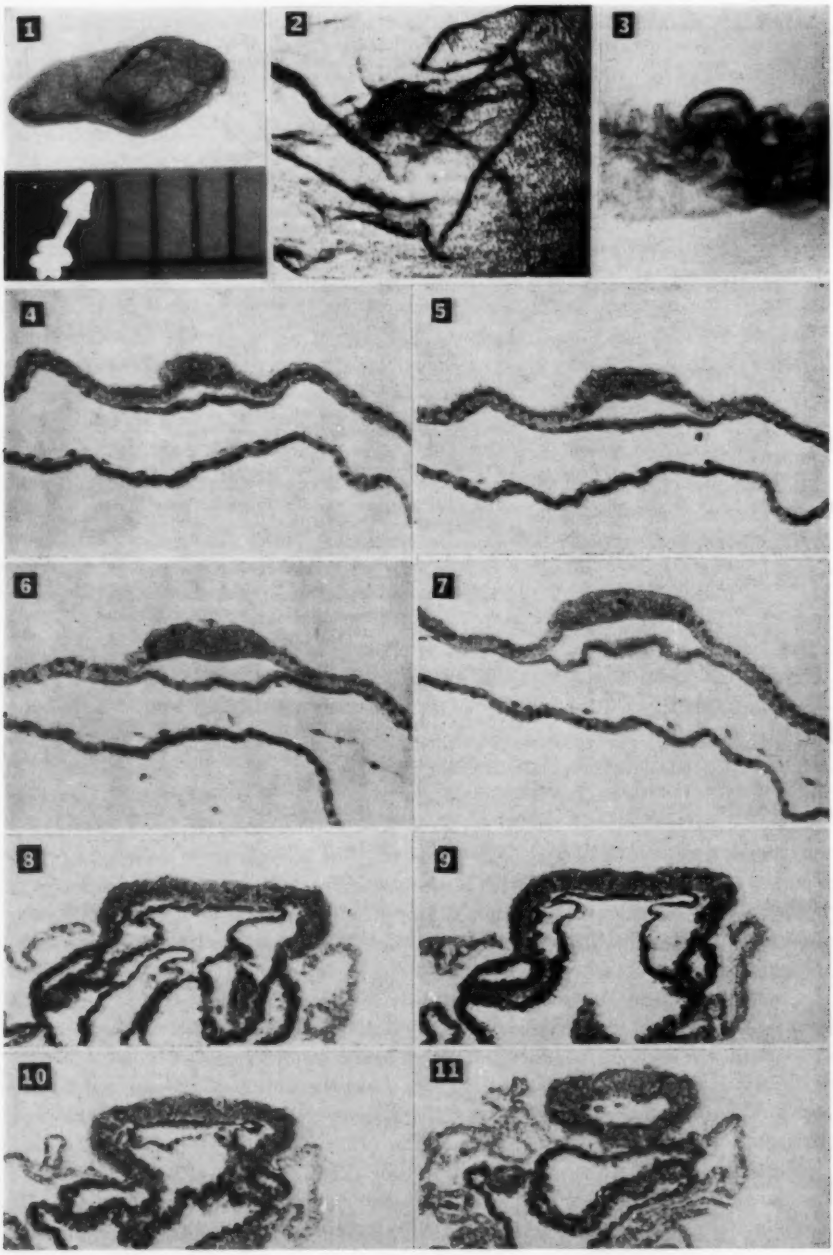

Plate 1

disk area and forms a complete lining for the blastocyst. Mitotic figures are seen frequently in the germinal disk and trophoblast, indicating a period of rapid growth and elongation.

Specimen NE-69 is shown in Figure 2. Although the chorionic vesicle was considerably longer than in the previous specimen, sections through the germinal disk indicated that the embryonic area did not differ significantly from NE-93. There was, however, a marked growth and folding of the wall of the trophoblast. The disk remained essentially spherical with a diameter of 0.35 mm. and was located in the mid-portion of the vesicle. There was no evidence of overlying trophoblast cells.

In Specimen NE-16 the trophoblast had elongated considerably, and the width of the chorion had increased to approximately 1.65 mm.

Figure 3 illustrates the prominent, raised, oval germinal disk of Specimen NE-132. The embryonic area was located approximately midway along the slender (1.5-mm. diameter) vesicle. Figures 8 to 11 are representative 10-micron sections taken at various levels through the germinal disk. This specimen shows a striking advancement over the specimen in Figures 4 to 7. The ectodermal disk is well-formed, and the mesoderm has separated to form the extraembryonic somatopleure, splanchnopleure, and coelom. Undercutting of the embryonic area indicates the formation of the future body stalk.

Although NE-22, when recovered, appeared to be the most advanced specimen of this 16-day-old group by virtue of its length, the embryonic disk was not discovered either upon gross examination or in tissue sections. Large numbers of pyknotic nuclei indicated that the trophoblast was undergoing degeneration, and it is reasonable to assume that the embryonic tissue proper had disappeared sometime prior to slaughter.

Seventeen-day embryos. Five embryos recovered 17 days after breeding were available for detailed study. The variation in appearance among the specimens was not as pronounced as it was on the previous day. The length of the chorion ranged from 70 to 240 mm., with a mean length of 144 mm. In all cases, the vesicle was somewhat enlarged in the central region, tapering toward the extremities. The embryo occupied a position in the approximate mid-portion of the vesicle, and at this stage there was a consistent tendency toward elongation of the germinal disk to assume an oval rather than a circular shape.

PLATE 1

1. Entire 16-day-old blastocyst NE-93 and millimeter rule photographed in saline. Arrow points to opaque embryonic disk. 6.2×.

2 and 3. Portions of elongate 16-day-old blastocysts, NE-69 and NE-132, showing characteristic raised germ disks on surface of trophoblast. 33× and 30×.

4, 5, 6, and 7. Transverse 8-micron sections of NE-93 (Figure 1) showing separation of endoderm layer from germ disk. Notice frequency of mitotic figures. Vestige of overlying trophoblast may be seen in Figure 6. 125×.

8, 9, 10, and 11. Transverse 10-micron sections of NE-132 (Figure 3). Note undercutting of embryonic area, the presence of a definitive yolk sac, and the delamination of mesoderm to form splanchnopleure, somatopleure, and extraembryonic coelom. 125×.

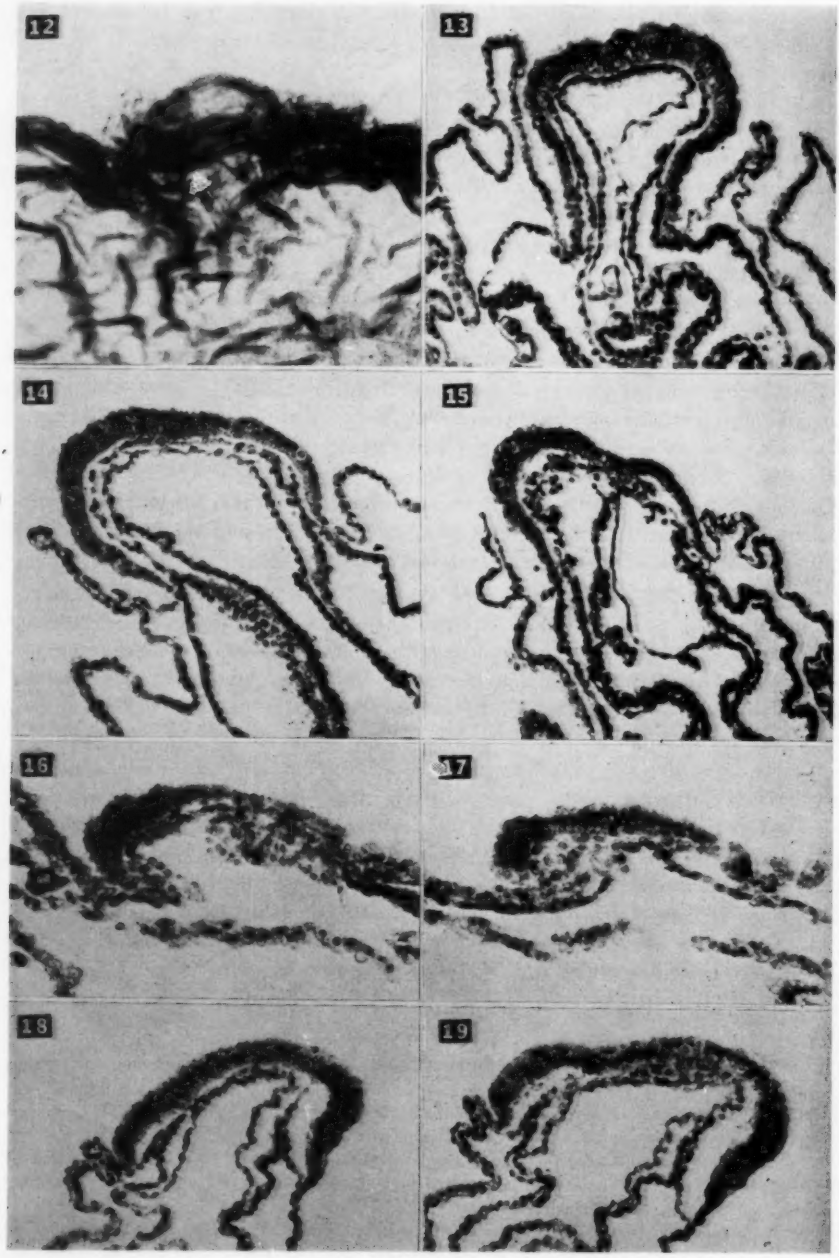

Plate 2

The embryonic area of the chorionic vesicle in specimen NE-50 was not positively identified either grossly or after serial sectioning. On the basis of microscopic examination of the tissue sections, it may be concluded that this specimen represents a case of persistent chorionic development in spite of prior embryonic failure. This conclusion is supported also by the relatively retarded length of the vesicle.

Specimen NE-61, whose germinal disk measured 0.6 by 0.8 mm., was located in the mid-portion of the vesicle. Sectioned at 8 microns, 98 sections were obtained. Aside from an over-all increase in the size of the disk, the appearance of the primary germ layers is essentially the same as in Specimen NE-132.

Figure 12 illustrates the gross appearance of Specimen NE-148. Sixty-eight sections were obtained at 10 microns and these revealed a well-developed mesoderm between the ectodermal plate and the yolk-sac endoderm. The lining of the cavity of the yolk-sac is composed of small, round cells with only a narrow rim of cytoplasm surrounding the oval, vesicular nuclei (Figures 13 and 14). The convex dorsal surface of the disk is rounded and smooth, except for evidence of a caudal primitive groove (Figure 15). The area of proliferation marking Hensen's node also may be seen in this photograph. The ectodermal cells with numerous mitotic figures rest on a basement membrane with the tall columnar cells gradually becoming cuboidal at the lateral borders of the disk. An abrupt transition to flat ectodermal cells is seen in the lateral body folds. There are indications that the embryonic disk has settled somewhat into the blastocyst, so that primitive amniotic folds appear to be growing centripetally over the embryo. If this interpretation is correct, this specimen is the most advanced of the group in terms of amnion formation.

The gross appearance of Specimen NE-39 illustrates the progressive cephalocaudal elongation of the disk, characteristic of this stage. Figures 16 and 17 represent sagittal sections through the embryonic area. Although the primary germ layers are well-established, there is no evidence of amniogenesis.

The chorionic vesicle of Specimen NE-55 extended the full length of the gravid horn. With the exception of NE-50, all other specimens in this age group occupied about two-thirds of the horn. At 10 microns, 105 serial sections were obtained, two of which are shown (Figures 18 and 19). In comparing these sections with those of NE-148 (Figures 13, 14, and 15), a marked similarity is noted.

PLATE 2

12. Mid-portion of 17-day-old blastocyst, NE-148, illustrating gross appearance of oval germinal disk and wrinkled trophoblast. 29×.

13, 14, and 15. Transverse 10-micron sections through NE-148. Numerous mitotic figures are seen among the tall columnar ectodermal cells of the disk. Evidence of a primitive groove in the caudal region is seen in Figure 15. 125×.

16 and 17. Representative sagittal sections of NE-39 illustrating cephalocaudal elongation of germ disk and well-defined primary germ layers. 125×.

18 and 19. Transverse 10-micron sections of NE-55. Note clear delineation of primary germ layers and well-defined separation of the mesoderm. The section in Figure 19 passes through the area of proliferation recognized as Hensen's node. 125×.

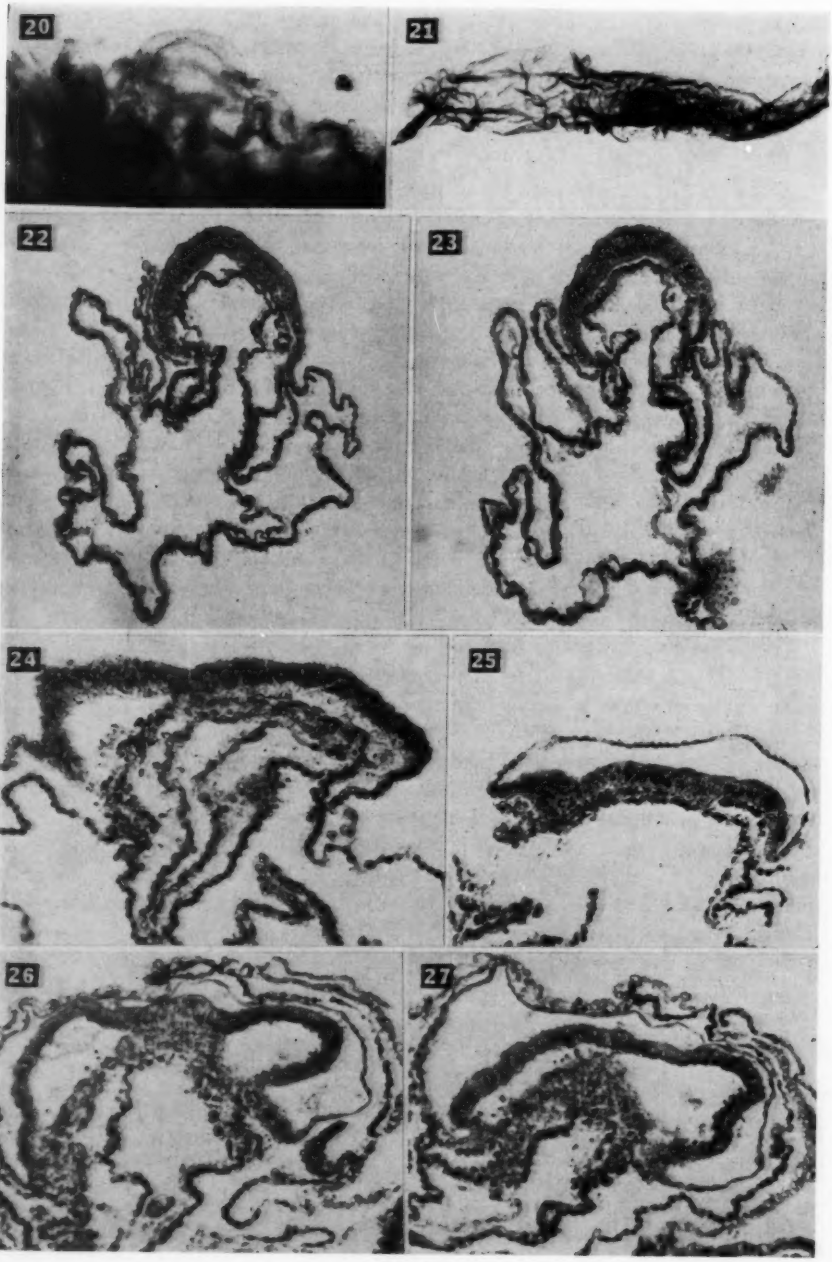

Plate 3

The former, however, appears to have progressed further, particularly with reference to a more pronounced Hensen's node and a more definitive mesoderm.

Eighteen-day embryos. Observations were made on five embryos recovered 18 days after the last breeding. In this age group, Specimen NE-49 appeared the least advanced. The embryonic disk (Figure 20) yielded 45 sections at 10 microns. These sections indicate that morphogenesis has not progressed appreciably beyond the stages previously described for 17-day embryos. The embryo displays a thickened ectoderm, a well-formed primitive streak and mesoderm, and continued extension of extraembryonic endoderm.

The gross appearance of Specimen NE-85 is seen (Figure 21). The embryo was located in the somewhat expanded mid-portion of the elongate, narrow chorionic vesicle. The embryonic disk measured 0.36 by 0.6 mm. after fixation, and 51 sections at 10 microns were obtained. Figures 22 and 23 are representative transverse sections through the embryo and vesicle, which show clearly the lateral portions of the mesoderm partially filling the area between the trophectoderm and yolk sac. The mesoderm has undergone delamination to form extraembryonic coelom. In the region where the heart will develop, this formation foreshadows the early appearance of pericardial coelom. As noted earlier in the 17-day-old Specimen NE-148, there is a suggestion of primitive amniotic folds. However, somatic mesoderm is not associated with the folded trophectoderm.

A definite primitive groove was grossly discernible in Specimen NE-90. This was confirmed in sections (Figure 24) with 56 somewhat oblique serial sections obtained at 10 microns. The continued elongation of the chorionic vesicle to fill the gravid horn and the progressive extension of the limits of the extraembryonic coelom placed this embryo in a stage of development beyond those previously described. Evidence for the upward bending of somatopleure at the periphery of the embryonic plate was entirely lacking, however.

When Specimen NE-37 was flushed from the horn of the uterus, examination of its entire length failed to produce a positive identification of the embryonic area. Representative portions of the central region were preserved, however, and in subsequent sectioning the embryo was located. Complete serial sections were not available for study, because the embryo had suffered some damage in

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PLATE 3

20 and 21. Gross appearance of 18-day-old germ disks of NE-49 and NE-85. Note progressive tendency for elongation of embryonic area. Chorionic vesicle remains slender, as seen in Figure 21. 25× and 15×.

22 and 23. Transverse sections of NE-85 (Figure 21) through the entire blastocyst in the embryonic area. Note the complete endodermal lining of the blastocyst, the lateral extensions of mesoderm, and the suggestion of early amniogenesis. 100×.

24. Oblique section through the primitive groove of NE-90. Note well-defined yolk sac and mesodermal elements. 125×.

25. A near-sagittal section of NE-37. Prominent features are the completed amnion, Hensen's node, primitive groove, and mass of notochordal cells. 100×.

26 and 27. Transverse sections of NE-131. The embryo is seen to be completely enclosed within the amniotic sac. Figure 26 is a section passing through Hensen's node; whereas, Figure 27 shows the thickening of the lateral mesoderm. 125×.

processing. The plane of the sections also passed somewhat obliquely through the longitudinal axis. The most notable feature of this embryo (as seen in Figure 25) is a well-defined, complete amniotic sac enfolding the developing embryo. Anterior to the thickened area of Hensen's node and the primitive groove is seen the rod-shaped mass of notochordal cells occupying the space between the ectoderm and endoderm.

Additional evidence for possible amnion formation during the 18th day of development was provided by Specimen NE-131. The embryonic area measured approximately 0.4 by 0.65 mm. after fixation, and a total of 74 transverse sections of 8 microns was obtained. This specimen was the most advanced in the series, judging by the microscopic appearance of the embryo and the extension of the chorionic vesicle into the nongravid horn. Figure 26 is a section through Henson's node showing the nodal area as a center of proliferation for a median band of cells, which seemingly merge with the hypoblast below. Fusion of the amniotic folds above the mid-dorsal region of the embryo completes the amniotic sac, and the embryo in its amnion lies free in the surrounding trophoderm. The thickened lateral mesoderm seen in this figure (and in Figure 27) foreshadows the emergence of the epimeric mesoderm and the future somatic mesoderm of the trunk area.

DISCUSSION

The determination of the exact age of recovered embryos is very difficult in most species, because of a lack of precise knowledge of the actual time of ovulation and the time of fertilization. The copulation age generally employed is, at best, an approximation and accounts for many of the discrepancies found in the literature. Thus, Winters *et al. (30)* dated the specimens from 6 P.M. of the day the cow was mated until the time of slaughter, and described a 13-day, 14-hr. embryo that was very similar to the 16-day-old specimen NE-93 of the present series. Chang *(5)* described similar embryos recovered from slaughterhouse material and, based on the description of Winters *et al. (30)* and Hamilton and Laing *(12)*, estimated their ages at from 13 to 14 days.

Similarly, the variation in development encountered within a single day among the specimens described in the present paper emphasizes the shortcomings of copulation-age estimates. This lack of precise information relative to the age of specimens makes it difficult to evaluate the influence of other factors, such as individuality, age, and breed differences.

Whereas the utimate aim of descriptive embryology is to characterize the successive and progressive developmental events as a continuum, the scarcity of accurately timed bovine material necessitates the establishment of standards within the limits of normal variation encountered using an arbitrary time-scale. The stage of embryonic development under discussion is one of extremely rapid growth and differentiation. In view of the heavy losses reported for repeat-breeders during the early stages of pregnancy, and the current interest in the causes of early embryonic mortality, suitable standards of normal bovine development expressed in terms of copulation age are needed.

The bilaminar condition of the growing germ disk described for the youngest specimen of the 16-day group is approximately comparable to the human embryo at the end of the second week *(17, 18)*, to that of the pig at about 8 or 9 days of age *(19)*, and to the sheep at from 10 to 11 days *(8)*. In the human, however, the amnion is established precociously by cavitation at from 7 to 8 days; whereas, in these other species, a more leisurely dorsal folding of the somatopleure occurs.

The more advanced specimens within the 16-day group are actively engaged in the gastrulation process. The growth and elongation of the blastocyst occurs soon after the endoderm has been established as a definite layer. The variation in the length of the chorionic vesicles noted in these specimens is often encountered within a single litter in the pig and attests to the rapidity of growth at this stage. Hawk *et al. (16)*, reporting on 31 bovine embryos recovered at 16 days, noted that the extraembryonic membranes varied in length from 2 to 225 mm., with a mean length of 95 mm. The disk itself lengthens cephalocaudally, converting the disk from a spherical to an oval shape. Both formative mesoderm and extraembryonic mesoderm make their appearance at this time, with the latter giving rise to the mesoderm of the extraembryonic somatopleure, splanchnopleure, and coelom. These features agree closely with the description by Winters *et al. (30)* of a 16-day, 14-hr.-old embryo.

The 17-day-old group is characterized by a continuing elongation of the blastocyst and local germ disk differentiation to establish the longitudinal axis of the embryo and the primitive streak. This stage of development is comparable to the 11- or 12-day-old pig, and the 13-day-old sheep, embryo. As might be expected, there is some overlapping in development when individual specimens are compared with the more advanced of the 16-day-old embryos, on the one hand, and the less developed of the 18-day-old specimens, on the other.

The most notable features of the 18-day-old group are the formation of the primitive groove, the extension of the boundaries of the mesoderm, the formation of the primitive node, the emergence of the notochord and, in some instances, the establishment of a definite amniotic sac. Although the successive steps in amnion formation are not clearly defined in these specimens, it is reasonable to assume a dorsal projection and fusion of anterior, lateral, and posterior amniotic folds similar to those found in the sheep and pig. With the attainment of the amnion and primitive streak stage, the 18-day-old cow embryo approximates the human embryo of the same age *(20)*, a 13- to 14-day-old sheep *(8)*, and a 13-day-old pig *(26)*. A suggestion that amnion formation in the cow is more precocious than in the pig derives from the contention of Heuser and Streeter *(19)*, that in the pig the embryonic area remains entirely exposed until after the primitive streak and neural folds are formed.

In the present series of 16 specimens, there are two instances of possible embryonic failure. Studies on the nature of reproductive failures in repeat-breeder cows have produced estimates that from 54 to 65% of all fertilized ova in such cows die before 34 days *(3, 4, 28, 29)*. In a similar study with a group of 80 repeat-breeder cows, Hawk *et al. (16)* estimated embryonic mortality be-

tween 16 and 34 days to be 51.7%, and suggested that the majority of the deaths occurred before 25 days.

It is undoubtedly significant that this period of high embryonic death coincides with these critical phases of morphogenesis during which the primary germinal tissues are established. These orderly, successive, integrated processes are dependent upon successful completion of each preceding step and a proper environment. Disturbing factors that in any way upset these normal interrelationships would preclude the successful continuation of the development of body form.

The expansion of the extraembryonic endoderm and the rapid growth of the trophoblast suggest that this period is a critical one in the nutrition of the embryo. The relatively late implantation (33 to 36 days) of the bovine embryo *(7, 22)* necessitates such a structural adaptation for the absorption of nutrients from the uterine environment after its own intrinsic energy sources have been exhausted. The functional state of the endometrium, in turn, depends upon maintenance of the corpus luteum, which at this same period in a normal estrous cycle would be undergoing degenerative processes. The stimulus for the continuation of a functional corpus luteum of pregnancy undoubtedly arises in the embryo and is probably of trophoblastic origin. Any consideration of possible causes of embryonic death, therefore, must take into account the complex interrelationships of the embryo, the uterus, and the gonads.

Then too, the numerous, intricate processes involved in gastrulation and primitive streak formation provide many opportunities for constitutional defects or fortuitous errors in development to express themselves in embryonic failures. These factors, in addition to the possibilities of endocrine and environmental causation of embryonic pathology, make accurate diagnosis relative to the nature and cause of individual failures virtually impossible with our present knowledge. A prime requisite for a final understanding of the problem is sufficient comprehensive information concerning the normal early embryology of the species to facilitate the recognition of abnormal and retarded development.

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INFLUENCE OF SEMINAL VESICULAR FLUID ON MORPHOLOGY OF BULL SPERMATOZOA[1]

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SUMMARY

This study presents the distribution of spermatozoa with protoplasmic droplets in the epididymides of slaughterhouse bulls. There were 11 paired and nine single epididymides included in the study. The mean per cent of spermatozoa with droplets in the caput, corpus, and cauda were 62, 41, and 84, respectively. The per cent of spermatozoa with droplets from the right and left sides of the reproductive tract of the same bull showed less variation than among bulls. Six per cent of the spermatozoa from the ampullae had droplets.

Fluids from the seminal vesicles, when added to epididymal spermatozoa, produced changes in the per cent of spermatozoa with protoplasmic droplets. The per cent of spermatozoa with distal protoplasmic droplets from the corpus and the cauda epididymis and the per cent of sperm with proximal droplets from the caput were decreased significantly ($P < 0.01$). The per cent of spermatozoa with distal droplets in the caput was increased significantly. The decrease in the per cent of total sperm with droplets after the addition of the seminal vesicular fluid was statistically significant ($P < 0.01$).

A round protoplasmic droplet is a structure normally observed on the epididymal spermatozoa of the bull. Attention was first drawn to this structure by Kölliker *(15)*, in 1856. Since then a number of workers have reported on its morphological origin *(6-10)*. The literature on the subject was summarized by McKenzie and Berliner in 1937 *(19)* and by Gresson in 1951 *(8)*. A majority of the reports agree that during spermatogenesis, after the elimination of the residual cytoplasm, a protoplasmic droplet, containing argentophil (Golgi) granules, is present immediately posterior to the head of the spermatozoon. Investigators have observed that spermatozoa after their entry into the epididymis possess the droplet in close proximity to the head, and that the droplet migrates along the mid-piece and the tail of the sperm during their passage through the epididymis and the ductus deferens *(3-5, 9, 12, 17, 22)*. Selivanova in 1937 *(22)* made a detailed study of the position of the droplet on spermatozoa from different parts of the reproductive tract of a number of farm animals. In two different parts of the caput of the bull she observed 23 to 67%, in the corpus 41 to 48%, and in the cauda 45 to 49%. She stated that the droplet shifted during the spermatozoon's passage from the caput into the cauda, and that the droplet was stretched along the mid-piece during the cell's sojourn in the corpus epididymis.

In 1947, Branton and Salisbury *(4)* also recorded the percentage of spermatozoa with droplets. They studied six bulls and obtained the following values for the spermatozoa with droplets: caput epididymis $80.32 \pm 6.79\%$, cauda

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69.90 ± 2.4%, and ampulla 23.19 ± 6.13%. Differences among bulls were not observed. Rao and Hart *(20)* gave similar percentages for cells taken from the cauda epididymis. Largerlöf *(16)*, from his extensive studies on impaired reproduction of bulls, concluded that the presence in the ejaculate of spermatozoa with droplets on their necks signified a reproductive disturbance. Hancock *(11)* has observed an unusually large number of sperm with droplets in infertile Guernsey bulls. Both Holt *(13)* and Mann *(18)* suggest that spermatozoa might use the droplet as a source of energy during the epididymal passage.

Normal ejaculates contain few spermatozoa with droplets *(1)*. A variety of solutions can cause detachment of the droplet, but the number of detachments is never as large as in normally ejaculated spermatozoa.

Selivanova *(22)* performed a large number of experiments in which she showed that seminal secretions and other solutions, as well as the rate of dilution, caused droplet detachment. Similar results were obtained by Rao and Hart *(20)*. Hancock *(12)* collected boar semen in separate 20-ml. fractions. In the first 20-ml. fraction, the number of sperm with the droplet was as high as in the ductus deferens. Subsequent fractions had a greatly reduced number of sperm with droplets. From this, Hancock concluded that the accessory gland secretions caused the detachment of droplets.

EXPERIMENTAL PROCEDURE

Bull reproductive tracts were obtained from the local meat-packing plant. The epididymides were separated from the testicles. There was some variation in lapsed time from the slaughter of the bulls until the different examinations were made. These examinations were usually completed within 2 to 3 hr. after slaughter. An incision was made in the middle of the caput, corpus, and cauda, and a small drop of semen was obtained by squeezing the epididymis near the incision, for studies on the distribution of sperm with droplets and on the position of the droplet of the spermatozoon. The caput incision was made on the side toward the corpus epididymis. Semen was obtained from the ampulla by stripping. Immediately, the semen was thoroughly mixed with eosin-nigrosin stain *(24)*. A uniform smear was prepared on a microscope slide. The slide was air-dried and the oil immersion objective was employed for observation. Three hundred spermatozoa were counted at random and the distribution of droplets recorded. The droplet was designated as proximal when situated on the neck and as distal when situated at the posterior end of the mid-piece of the sperm cell.

In another series of experiments, the influence of the fluids from seminal vesicles on the protoplasmic droplet was investigated. Seminal vesicular fluid was obtained by compressing the excised seminal vesicles and catching the fluid. On several occasions some admixture of blood was observed. The experimental drop of epididymal spermatozoa was placed in a small bacteriological culture tube to which 0.5 ml. of seminal vesicular fluid was added. Five minutes later, a drop of this mixture was stained with eosin-nigrosin and a differential count was performed as described previously. Control samples were treated in the same

manner as samples for the relative distribution studies. The significance of the changes caused by the seminal vesicular fluid was analyzed by the paired *t*-test; for the purpose of analysis all of the per cent values were converted to angles *(23)*.

RESULTS

In Tables 1 and 2, values recorded on the same line represent the caput, corpus, and cauda from the same epididymis. Some of the specimens were badly mutilated by the packinghouse workers so that some parts of the epididymis could not be used. This accounts for the missing values. The data in this study represent 11 paired epididymides and nine individual ones.

The distribution of droplets on spermatozoa from the different parts of the epididymides of bulls is shown (Table 1). Spermatozoa with droplets in the

TABLE 1

Percentage of spermatozoa with protoplasmic droplets and the position of the droplet as influenced by epididymal location

Epididymis number	Caput Epididymis		Corpus Epididymis		Cauda Epididymis	
	Distal droplet	Proximal droplet	Distal droplet	Proximal droplet	Proximal droplet	Distal droplet
	(%)					
1a[a]	9	25	14	0	92	0
1b	9	27	11	0	90	1
2a	7	24	20	0	84	1
2b	4	22	19	4	87	1
3a	0	85	37	1	79	3
3b	2	83	34	1	83	2
4a	36	30	48	1	76	1
4b	33	28	42	2	81	1
5a	27	30	69	3	81	1
5b	24	28	62	6	80	0
6a	6	56	43	2	81	2
6b	5	54	40	2	81	1
7a	14	52	38	0	82	5
7b	17	51	34	0	64	23
8a	22	44	39	5	90	0
8b	22	41	38	4	85	0
9a	7	39	40	4	73	2
9b	8	37	53	4	69	1
10a	0	80	25	0	79	2
10b	0	84	22	0	89	0
11a	9	61	29	0	....	....
11b	10	61	30	3	....	....
12	15	70	61	3	79	3
13	13	76	74	7	90	2
14	8	50	59	6	97	0
15	0	82	61	0	85	2
16	16	66	46	0	86	0
17	2	29	59	0	88	1
18	8	75	54	1	69	0
19	....	....	....	....	88	0
20	....	....	....	....	87	0
21	11	72	18	2	....	....
Range	0–36	22–85	11–74	0–7	64–97	0–23
Mean	9.3	52.4	40.0	1.0	83.2	1.0
Standard deviation	9.59	21.35	16.18	2.44	7.28	4.24

[a] The numbers 1a, 1b, etc. indicate these epididymides were from the same pair of testicles. A single number indicates that only one epididymis from a pair of testicles was used.

TABLE 2

Percentage of spermatozoa with protoplasmic droplets from various parts of the epididymides and the effect of seminal vesicular fluid on the droplet

Epididymis number[a]	Caput Epididymis				Corpus Epididymis				Cauda Epididymis			
	Position of droplet				Position of droplet				Position of droplet			
	Distal		Proximal		Distal		Proximal		Distal		Proximal	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
	(%)											
12	15	16	70	61	61	54	3	3	79	69	3	3
13	13	11	76	67	74	47	7	10	90	83	2	0
14	8	16	50	36	59	33	0	0	97	70	0	0
15	0	4	82	57	61	42	0	0	85	43	2	3
16	16	24	66	32	46	22	0	0	86	43	0	0
17	2	7	29	8	59	44	0	0	88	44	1	4
18	8	17	75	42	54	34	1	1	69	72	0	0
19	....	....	....	....	....	....	....	....	88	38	0	2
21	11	20	72	32	18	22	2	1	....	....	....	....
Mean	9.1	14.4	59.0	41.8	54.0	37.2	1.6	1.9	85.4	56.3	0.9	1.3
	$P < 0.01$		$P < 0.01$		$P < 0.01$				$P < 0.01$			

[a] The epididymis numbers correspond to the ones in Table 1.

caput epididymis comprised 61.7% of the total spermatozoa; this value is within the range of values quoted by Selivanova *(22)*, but is lower than the value of 80.32% observed by Branton and Salisbury *(4)*. Forty-one per cent in the corpus epididymis had droplets. This value corresponds to the value given by Selivanova *(22)*. The number of spermatozoa possessing droplets in the cauda was 84%, and this value is higher than the ones observed by other investigators. Branton and Salisbury *(4)* obtained the value of 62.9% and Rao and Hart *(20)* observed the range of from 18 to 75%. In the caput epididymis, spermatozoa with proximal droplets predominated; in the corpus and in the cauda epididymis spermatozoa with distal droplets predominated. These observations are in agreement with published reports *(4, 22)*.

The data (Table 2) show the influence of seminal vesicular fluid on the droplet position on spermatozoa from different parts of the epididymis. Addition of seminal vesicular fluid to spermatozoa from the caput caused a highly significant decrease ($P < 0.01$) in the per cent with proximal droplets. In the corpus and in the cauda epididymis the per cent of spermatozoa with distal droplets was decreased significantly ($P < 0.01$) by the addition of fluids. The addition of seminal vesicular fluids to sperm samples from the three parts of the epididymis caused a highly significant ($P < 0.01$) decrease in the total number of spermatozoa with droplets.

Ninety-four per cent of spermatozoa from nine ampullae had no droplets. The distribution of droplets on spermatozoa containing them was as follows: distal—from 1 to 19%, proximal—from 0 to 11%.

DISCUSSION

Differences between relative distribution values observed during this study and those observed by other investigators could stem from several sources. First, part of the difference could be accounted for by the inherent bull differences, though Branton and Salisbury *(4)* did not observe such differences in their study of six bulls. Second, the possible effect of the stain used on the droplet must be considered. This effect was not tested but, from other information available *(20, 22)*, one could infer that the stain might cause droplet translocation and disappearance. Third, no breeding records were available for these slaughterhouse bulls and the possibility of spermatogenic disturbances can not be discounted.

The values obtained for the paired sections (Table 1) show that within the same animal the two sides of the reproductive tract show similar distributions. This could indicate approximately the same maturation rate, or that the evacuations for the two sides were about equal.

The percentage of cells possessing the distal cytoplasmic droplet in the cauda was more uniform (range of from 64 to 97%) than the percentage of cells containing distal droplets (range of from 11 to 74%) in the corpus, or the proximal droplets in the caput (range of from 22 to 85%). It may represent a more uniform success of sectioning for the cauda than for the corpus or the caput, or that the spermatozoa from the cauda may respond more uniformly to the stain.

If the droplet is a cytoplasmic remnant containing Golgi granules, as the majority of investigators claim *(8-10)*, then droplets should be present in larger numbers on spermatozoa from the caput. Hancock *(11)* observed a progressive decrease in the number of droplets from the beginning to the end of the caput. It is unlikely that the droplet is acquired from epididymal secretions, although Gatenby and Collery *(6, 7)* have indicated that epididymal secretory granules can become attached to the spermatozoa. The number of epididymal spermatozoa without the droplet was largest in the corpus epididymis, rather than the cauda. This has been explained by Selivanova *(22)* as being the result of the stretching out of the droplet along the mid-piece. On occasion, thickening of the mid-piece was observed on spermatozoa from the corpus. Whether this was caused by stretching of the droplet can not be answered by this study.

Another important question raised by the presence of the droplet on spermatozoa is that of the maturity of the sperm. A number of authors *(11, 16, 21)* have stated that presence in an ejaculate of a number of spermatozoa with droplets indicates lowered fertility. Such statements are not wholly convincing, since the bulls under consideration produced ejaculates with a high count of abnormal sperm. Lagerlöf *(17)* modified his view and considered only spermatozoa with proximal droplets as possible indicators of sperm immaturity.

The absence of the droplet from ejaculated sperm is owing either to a mechanical or to a chemical factor, or both, operating during the transition from the epididymis into the ejaculate or the ampulla. The results (Table 2) show that the seminal vesicular fluid can cause a significant reduction in droplet number. In this respect, these data agree with the findings of Selivanova *(22)* and of Rao and Hart *(20)*.

Most of the spermatozoa found even in the first ejaculate come from the cauda *(2)*, a portion of the tract where droplets are numerous. Their absence in the ejaculate indicates a rapid disappearance of the droplet under the influence of ejaculation, rather than a rapid maturation, as suggested by some authors. Exhaustion trials *(2)* did not reveal increased numbers of sperm with droplets in consecutive ejaculates, and their results agree with the observation of Kirillov *(14)* in this respect.

In one occluded ductus deferens, the spermatozoa from the cauda were without droplets, which suggests a ripening process similar to the one described by Young *(25)*. On another occasion *(2)*, when ten successive ejaculates were taken from a bull, all the ejaculates had a high proportion of cells with droplets. Prior to this exhaustion, collections were taken from this bull approximately once a week. The usual influence of ejaculation must have been disturbed in this case, since on other occasions the ejaculates from this bull were free of spermatozoa with droplets.

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VALUE OF ADDING CORN SILAGE AND LIMITED QUANTITIES OF HAY TO A GRASS SILAGE, LIMITED GRAIN RATION FOR DAIRY HEIFERS[1]

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SUMMARY

Twelve Holstein and 12 Guernsey heifers were used to determine if dairy herd replacements can be raised satisfactorily from a few days to 2 yr. of age on a commercial milk replacement, 500 lb. concentrates and one or more of the following forage combinations: grass silage alone, grass silage and corn silage (50-50), grass silage and limited hay, and grass silage and corn silage (50-50) and limited hay. The silages were fed ad libitum and hay was fed at the rate of ¾ lb. per 100 lb. body weight per day. The inclusion of hay in the ration increased body weight gains, but the feeding of corn silage decreased gains. These results were due to differences in the consumption of nutrients from the various forage combinations. The Guernseys ate approximately the same total amount of forage as the Holsteins; thus, generally carried more body condition. The animals fed grass silage and hay averaged 94% of normal at 2 yr. The other groups did not make satisfactory gains. The feeding of corn silage had no effect on conception rate, but animals fed hay required more services per conception than those not fed hay. Groups of animals which made poor gains generally were delayed in becoming sexually mature and with-calf.

Because of the relatively large number of replacements that must be raised to maintain a dairy herd and to permit culling, anything that can be done to reduce the cost of feeding such animals is of considerable importance. The most promising way to do this appears to be to replace a maximum part of the concentrates in the ration with lower-cost nutrients from high-quality forage. With the increased use of grass silage as a means of obtaining early harvesting and low field losses owing to weather damage, it appeared that forage preserved in this way might be particularly useful in feeding dairy herd replacements when limited amounts of concentrates are used.

Sykes *et al. (8)*, in an experiment with Holstein and Jersey heifers, fed limited milk and grain with high-quality forage in the following forms: alfalfa hay, alfalfa silage plus alfalfa hay, and alfalfa silage. They obtained normal growth with alfalfa hay but not with alfalfa silage fed as the sole forage. When a limited amount of hay was fed with the alfalfa silage, the growth rate was improved but did not equal that obtained on hay. In continuation of this work, Thomas *et al. (9)* found that the feeding of equal parts of corn silage and alfalfa silage did not increase gains over alfalfa silage alone. In these two studies, observations were made to 2 yr. of age and differences among groups receiving the

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various forages were not apparent until past 5 mo. of age. Porter and Kesler *(5, 6)* fed calves a limited amount of starter and either grass-legume silage or hay in one experiment and alfalfa silage or a comparable hay in another. These experiments, which continued until the calves were 112 days of age, did not reveal any difference between the silages and the hays.

Sherwood and Jones *(7)* reported that the extra gains in dairy heifers resulting from supplementing a ration of good-quality alfalfa hay with grain were small and did not justify the cost of the grain. Headley *(3)* fed all-roughage rations to older heifers and found that similar gains were obtained from alfalfa hay and alfalfa hay supplemented with alfalfa silage. Also working with older heifers, and limiting grain to 3 lb. per day, Wilson and Bartle *(10)* obtained only slightly lower growth rates when alfalfa silage was fed in comparison to comparable alfalfa hay. In earlier work at the New Hampshire Agricultural Experiment Station *(4)*, yearling heifers fed 2 lb. of grain per day made better gains when legume-grass silage was included in the ration than when comparable hay was fed. Converse and Wiseman *(2)* fed animals from birth through one lactation, using corn silage as the sole forage. Normal growth and development were obtained.

The study reported here was carried out to determine if an early-cut grass silage could be used successfully in feeding dairy heifers from a few days to 2 yr. of age, along with limited quantities of grain; also, to determine how the addition of corn silage and/or limited hay to such a ration would affect nutrient consumption and body weight gains.

EXPERIMENTAL PROCEDURE

Twelve Holstein and 12 Guernsey heifer calves purchased from nearby farms were used as experimental subjects. They were placed on the experiment at a few days of age. The calves were kept in individual pens and all feed was weighed. They were fed a commercial milk replacement according to the manufacturer's recommendations. Beginning as soon as the calves would eat it, a commercial 20% crude protein dry calf starter was fed at a level that the animals would clean up, until a maximum of 2½ lb. per day was being consumed. As each calf consumed a total of 200 lb. of this mixture, it was replaced abruptly by a 16% crude protein concentrate feed, mixed according to the New England Dairy Feed Conference formula for 1953, with the maximum daily concentrate consumption remaining at 2½ lb. per day. As the total grain consumption per animal approached 500 lb. (200 lb. starter and 300 lb. 16%), it was gradually decreased and discontinued at this amount. The age at which this happened usually was between 9 and 10 mo.

The animals were allowed access to trace-mineralized salt blocks in their stalls and a mixture of equal amounts of trace-mineralized salt and dicalcium phosphate in the exercise lot. They were permitted exercise as weather permitted. Irradiated yeast was fed to insure an adequate intake of vitamin D.

As the animals were placed on the experiment they were assigned to a particular forage combination by randomization. This resulted in three animals of

each breed being fed each of the following forage combinations: grass silage ad libitum, grass silage ad libitum and hay at the rate of ¾ lb. per 100 lb. body weight per day, grass silage and corn silage (50-50) ad libitum, and grass silage and corn silage (50-50) ad libitum and hay fed at the rate of ¾ lb. per 100 lb. body weight per day. The feeding of these forage combinations was started as soon as the calves would eat forage and continued through a period of 2 yr.

The grass silages used were early-cut mixtures of timothy, bromegrass, and native grasses containing relatively small proportions of Ladino clover and lesser amounts of other legumes. For the most part, the grass silages were preserved by the wilting method, but a few lots were preserved with sodium metabisulfite. Quality varied, as on most dairy farms. The corn silages fed were a fairly early-maturing variety harvested in the glaze stage. They could be classified as average in quality. The hays used were early-cut, field-baled mixtures similar in composition to the grass silages.

The heifers were bred artificially by the local technician, using semen selected by him. The breeding of the Guernseys was started on the first heat period after they had reached a body weight of 600 lb., or at the age of 18 mo., whichever came first. The comparable values for Holsteins were 750 lb. and 18 mo.

RESULTS AND DISCUSSION

The average body weights and heights at withers by 6-mo. periods for the animals receiving the various forage combinations are given (Table 1). These values also are given as a percentage of the Morrison normals. Table 2 gives the average forage consumption of the various groups by periods. Table 3 gives nutrient consumption from forage of the animals in the various groups in terms of crude protein and gross energy. These values were used because they are accurately and easily determined and eliminate the errors resulting from the drying of silage for analysis *(1)*. Table 4 shows a summary by periods of the effect of hay and corn silage supplements on body weight; whereas, Table 5 shows the effect of these supplements on protein and energy consumption.

Statistical analysis of the data for the 2-yr. period showed that there was a highly significant increase in growth when a limited amount of hay was added to the silage rations, and a highly significant decrease in growth when corn silage was added to the grass silage or grass silage plus hay rations. It further showed that the addition of hay increased energy consumption and that the feeding of corn silage decreased it. Both of these effects were highly significant. Similar effects on protein consumption were found.

When all of the data were combined, these effects amounted to increases of 16.0% in body weight gain, 20.3% in energy consumption, and 23.8% in protein consumption, owing to the addition of a limited amount of hay to the ration. When corn silage was added to the ration, the decreases amounted to 15.0% in body weight gains, 28.0% in energy consumption, and 30.5% in protein consumption. These results indicate simply that growth must be supported by adequate nutrient intake and that the nutrient intake was determined by the amount of the different forages that the heifers would eat. It does not indicate

TABLE 1

Average body weights and heights at withers and per cent of Morrison normal at end of each period

Forage combination	6 mo.				12 mo.				18 mo.				24 mo.			
	Body weight		Height at withers		Body weight		Height at withers		Body weight		Height at withers		Body weight		Height at withers	
	(lb.)	*(%)*	*(in.)*	*(%)*	*(lb.)*	*(%)*	*(in.)*	*(%)*	*(lb.)*	*(%)*	*(in.)*	*(%)*	*(lb.)*	*(%)*	*(in.)*	*(%)*
								Guernseys								
Grass silage	238	(89)	36.1	(97)	449	(92)	43.3	(100)	613	(93)	46.4	(100)	778	(95)	48.6	(101)
Grass silage + hay	235	(88)	36.3	(98)	448	(91)	43.5	(100)	620	(94)	46.8	(101)	797	(97)	49.3	(103)
Grass silage + corn silage	196	(73)	34.6	(93)	379	(77)	41.2	(95)	495	(75)	43.9	(95)	624	(76)	46.6	(97)
Grass silage + corn silage + hay	227	(85)	35.6	(96)	414	(85)	41.9	(97)	566	(85)	45.4	(98)	713	(87)	47.2	(98)
								Holsteins								
Grass silage	243	(64)	36.0	(91)	479	(70)	43.3	(94)	655	(74)	46.9	(94)	789	(72)	48.9	(94)
Grass silage + hay	295	(78)	37.3	(94)	566	(83)	45.0	(97)	828	(93)	48.6	(98)	1,006	(91)	50.5	(97)
Grass silage + corn silage	257	(68)	36.3	(91)	434	(63)	42.9	(93)	563	(63)	46.1	(93)	671	(61)	48.6	(94)
Grass silage + corn silage + hay	253	(67)	36.0	(91)	494	(72)	43.5	(94)	683	(77)	46.8	(94)	822	(75)	49.3	(95)

TABLE 2

Average forage consumption by periods

Forage combination	0–6 mo.			6–12 mo.			12–18 mo.			18–24 mo.			Total 24 mo.		
	Grass silage	Corn silage	Hay	Grass silage	Corn silage	Hay	Grass silage	Corn silage	Hay	Grass silage	Corn silage	Hay	Grass silage	Corn silage	Hay
	(lb.)														
	Guernseys														
Grass silage	1,142			4,355			7,848			8,936			22,281		
Grass silage + hay	893		132	3,569		410	6,658		690	7,640		908	18,760		2,140
Grass silage + corn silage	376	364		1,753	1,476		3,525	2,529		4,423	3,176		10,077	7,545	
Grass silage + corn silage + hay	442	374	103	1,859	1,717	402	3,552	2,650	622	4,381	3,188	867	10,234	7,929	1,994
	Holsteins														
Grass silage	973			4,786			7,862			9,156			22,777		
Grass silage + hay	1,032		178	4,236		478	7,430		904	8,197		1,202	20,895		2,762
Grass silage + corn silage	515	462		2,027	1,821		3,812	2,814		4,544	3,127		10,898	8,224	
Grass silage + corn silage + hay	379	367	149	1,525	1,303	448	3,216	2,650	781	3,997	2,119	962	9,117	6,439	2,340

TABLE 3

Average crude protein and gross energy consumption by periods

Forage combination	0–6 mo.		6–12 mo.		12–18 mo.		18–24 mo.		Total 24 mo.	
	Protein	Energy	Protein	Energy	Protein	Energy	Protein	Energy	Protein	Energy
	(lb.)	*(T.)*	*(lb.)*	*(T.)*	*(lb.)*	*(T.)*	*(lb.)*	*(T.)*	*(lb.)*	*(T.)*
	Guernseys									
Grass silage	55	1,318	173	3,202	267	4,665	222	8,366	717	17,551
Grass silage + hay	58	1,299	178	3,612	302	5,237	258	8,813	796	18,961
Grass silage + corn silage	26	637	99	2,139	182	3,441	154	5,193	461	11,410
Grass silage + corn silage + hay	39	867	147	3,092	220	4,676	235	6,737	641	15,372
	Holsteins									
Grass silage	48	1,148	193	3,953	299	4,773	216	6,761	756	16,635
Grass silage + hay	70	1,548	218	4,502	363	6,229	294	8,738	945	21,017
Grass silage + corn silage	34	821	122	2,549	184	3,674	161	5,266	501	12,310
Grass silage + corn silage + hay	43	918	131	2,848	249	4,881	209	5,657	632	14,304

TABLE 4

Average body weights as influenced by hay and corn silage feeding

Forage additions	6 mo.		12 mo.		18 mo.		24 mo.	
	Body weight	Height at withers	Body weight	Height at withers	Body weight	Height at withers	Body weight	Height at withers
	(lb.)	*(in.)*	*(lb.)*	*(in.)*	*(lb.)*	*(in.)*	*(lb.)*	*(in.)*
				Guernseys				
Hay	231	36.0	431	42.7	588	46.1	746	48.3
No hay	217	35.4	414	42.3	554	45.2	701	47.6
Corn silage	212	35.1	397	41.6	531	44.7	685	46.9
No corn silage	236	36.2	448	43.4	616	46.6	786	49.0
				Holsteins				
Hay	274	36.7	530	44.2	756	47.7	914	49.9
No hay	250	36.1	457	43.1	609	46.5	730	48.8
Corn silage	255	36.2	464	43.2	623	46.5	746	49.0
No corn silage	269	36.7	523	44.2	742	47.8	898	49.7

that hay is a better feed than grass silage or that corn silage is an inferior feed. It means simply that animals fed under the conditions of this experiment had the preferences shown. These results are in good agreement with those of Sykes *et al. (8)*, as far as the value of limited hay is concerned. The results from corn silage are not in agreement with the ideas generally held.

One of the other unexpected observations made during the experiment was that the Guernseys generally grew at a more nearly normal rate than the Holsteins. This is explained by the fact that the Guernseys ate about the same total amount of protein and energy as the Holsteins during the 2-yr. period, even though the Guernseys were smaller and grew less during the experimental period. These results suggest that, contrary to general opinion, the smaller breeds may grow at least as well on high-forage rations as do the heavy breeds.

Table 6 shows the reproductive performance of the animals on the various treatments. These results indicate a rather unsatisfactory breeding record. The lowest average age at conception for any group of Holsteins was 22 mo. and the lowest average age for Guernseys was 20 mo. The highest average age at conception was nearly 26 mo. for Holsteins and 24 mo. for Guernseys. In general, age at conception was related to growth. Those animals which made the poorest gains became pregnant last.

The average number of services per conception for the different breeds and forage combinations varied from 1.67 to 3.33. There was no difference between those animals which received corn silage and those which did not. On the other hand, those animals which received hay required 3.17 services per conception, whereas those which did not receive it required only 1.9 services per conception. This difference was significant at the 5% level. The reason for this apparently adverse effect of limited hay feeding on reproduction is not known.

No trouble was experienced in getting the calves to eat grass silage as early as they would eat hay. Corn silage was eaten a little more reluctantly. The feeding of silage did not cause scouring except for a period between 4 and 8 wk.

TABLE 5

Average consumption of crude protein and gross energy as influenced by hay and corn silage feeding

Forage additions	0–6 mo.		6–12 mo.		12–18 mo.		18–24 mo.		Total 24 mo.	
	Protein	Energy	Protein	Energy	Protein	Energy	Protein	Energy	Protein	Energy
	(lb.)	*(T.)*	*(lb.)*	*(T.)*	*(lb.)*	*(T.)*	*(lb.)*	*(T.)*	*(lb.)*	*(T.)*
					Guernseys					
Hay	98	2,166	324	6,704	522	9,913	493	15,549	1,437	34,332
No hay	81	1,955	272	5,342	449	8,105	376	13,559	1,178	28,961
Corn silage	65	1,504	246	5,232	403	8,117	389	11,930	1,103	26,783
No corn silage	114	2,617	351	6,814	569	9,901	480	17,178	1,514	36,510
					Holsteins					
Hay	113	2,466	348	7,350	612	11,110	503	14,395	1,576	35,321
No hay	81	1,970	315	6,502	483	8,447	377	12,028	1,256	28,947
Corn silage	76	1,739	253	5,397	433	8,554	370	10,923	1,132	26,613
No corn silage	118	2,697	411	8,455	663	11,003	510	15,499	1,702	37,654

TABLE 6

Reproductive performance of animals on different forage combinations

Forage combination	Age 1st heat	At first service		Services/ conception	At conception	
		Age	Weight		Age	Weight
	(yr.)(day)	*(yr.)(day)*	*(lb.)*	*(No.)*	*(yr.)(day)*	*(lb.)*
			Guernseys			
Grass silage	1 — 169	1 — 233	656	1.67	1 — 249	672
Grass silage + limited hay	1 — 199	1 — 215	647	3.33	1 — 305	702
Grass silage + corn silage	1 — 269	1 — 327	600	1.67	1 — 341	605
Grass silage + corn silage + limited hay	1 — 179	1 — 257	628	3.00	1 — 358	781
Av.	1 — 204	1 — 258	633	2.42	1 — 313	690
			Holsteins			
Grass silage	1 — 167	1 — 297	748	2.00	1 — 342	748
Grass silage + limited hay	1 — 136	1 — 198	824	3.00	1 — 305	929
Grass silage + corn silage	1 — 183	1 — 332	652	2.50	2 — 22	652
Grass cilage + corn silage + limited hay	1 — 208	1 — 275	734	3.33	2 — 51	869
Av.	1 — 173	1 — 275	734	2.73	1 — 360	813

of age. This may have been at a time when rumen activity had not reached a normal state. Later in the experiment, feces were normally rather dry. The feeding of grain to other animals fed these same forages resulted in feces of a much softer nature.

From a growth standpoint, this study indicates that rather satisfactory results can be obtained from feeding dairy heifers on 500 lb. grain and liberal quantities of early-cut grass silage supplemented with limited amounts of good hay. In fact, Guernseys fed in this way averaged 97% of the Morrison normal at 2 yr. and the Holsteins, 91%. From a breeding standpoint, however, the results were not satisfactory. Several extra months were required to get the animals into production. This would represent a considerable loss to the dairyman because of the increased number of animals which would be required to maintain a herd. The number of services per conception was considerably higher than desired, particularly in those groups which received hay. This seems rather odd, because these animals generally grew more rapidly than those animals which did not receive hay and were larger when bred. This problem should be given further study.

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TECHNICAL NOTE

FACTORS AFFECTING THE ESTROGENIC CONTENT OF ALFALFA SILAGE[1,2]

The occurrence of estrogens in plants has been the subject of a recent review *(2)*. Presumably, factors responsible for differences in the estrogenic content of plants, such as species of plant or stage of maturity *(4)*, would also influence the estrogenicity of silage made from plant material. Additionally, it has been reported that molasses preservation of alfalfa silage increases its estrogenic activity *(3)*. The object of this study was to determine the effect of stage of maturity, type of preservative, and length of fermentation on the estrogenicity of alfalfa silage.

All silages were made by placing 2 lb. of freshly chopped, second-cutting alfalfa in plastic bags *(7)* and allowing fermentation to progress for varying intervals. In some instances, a fresh alfalfa sample was dehydrated for determination of original estrogenic activity. The silages were made either without preservative or with the addition of 80 lb. of molasses or 8 lb. of sodium bisulfite per ton of plant material. The estrogenic content of the silages was determined, using a modification of a previously described technique for the assay of plant estrogens *(3)*. Five mice were used to assay each silage sample, except in one instance when four mice were used. In the latter case a fifth statistic equal to the mean of the group was added for ease of statistical treatment. All data were treated for statistical significance by the method of analysis of variance *(5)*.

The data presented (Table 1) indicate that alfalfa silage had a higher estrogenic content than the control laboratory ration. Alfalfa cut in the bud or 1/10th-bloom stage is more estrogenic than that cut at later stages of maturity. These differences were highly significant ($P < 0.01$) (Table 2). Statistical treatment of the data presented (Table 3) revealed no significant differences between the various groups, indicating that under the conditions of this experiment, type of preservation or ensiling per se was without effect. This is in contrast to work previously reported *(3)*. It has been our experience that molasses preservation has a variable effect on the estrogen content of alfalfa silage.

Recent work indicates that strains of alfalfa vary widely in their natural content of estrogenic compounds *(6)*. One such compound isolated from alfalfa is coumestrol *(1)*. It is

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TABLE 1

Effect of stage of maturity and type of preservative on estrogen content of alfalfa silage

Stage of maturity	Type of preservative	Body wt.	Uterine wt.	Body wt.
		(g.)	*(mg.)*	*(%)*
Bud	None	26.40	18.68	0.071
	Molasses	23.20	36.80	0.159
	Sodium bisulfite	24.60	26.16	0.107
	Mean		27.21	
1/10th-Bloom	None	25.60	31.44	0.123
	Molasses	25.60	26.72	0.105
	Sodium bisulfite	26.20	35.88	0.136
	Mean		31.35	
1/2-Bloom	None	23.00	15.60	0.067
	Molasses	24.20	19.20	0.079
	Sodium bisulfite	24.60	20.92	0.086
	Mean		18.57	
Full-bloom	None	24.60	22.68	0.092
	Molasses	24.25	18.10	0.075
	Sodium bisulfite	25.60	24.88	0.098
	Mean		21.89	
Mature	None	26.00	25.04	0.097
	Molasses	28.20	22.60	0.081
	Sodium bisulfite	26.00	20.60	0.080
	Mean		22.75	
Control—laboratory ration		26.20	14.44	0.055

concluded that the estrogenic activity of alfalfa silage is more likely to be a function of original activity of the alfalfa than a result of the ensiling process.

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TABLE 2

Analysis of variance. Stage of maturity and type of preservative

Source	D. F.	Sum of squares	Mean square	F-ratio
Total	79	4,627.26		
Treatment	15	3,229.84	215.32	9.86[a]
Control vs. others	1	460.66	460.66	21.10[a]
Stage	4	1,487.41	371.85	17.03[a]
Preservative	2	116.60	58.30	2.67
Preservative × stage	8	1,165.18	145.65	6.67[a]
Error	64	1,397.42	21.83	

[a] $P < 0.01$.

TABLE 3

Effect of length of fermentation and type of preservative on estrogen content of alfalfa silage

Length of fermentation	Type of preservative	pH	Body wt.	Uterine wt.	Body wt.
(days)			*(g)*	*(mg.)*	*(%)*
	None	5.2	27.00	24.62	0.092
2	Molasses	4.6	24.20	22.52	0.093
	Sodium bisulfite	5.6	28.20	23.72	0.084
	None	4.6	25.60	22.76	0.089
4	Molasses	4.3	29.00	24.60	0.085
	Sodium bisulfite	5.5	27.40	23.20	0.084
	None	4.8	26.20	24.76	0.095
8	Molasses	4.2	25.80	24.68	0.096
	Sodium bisulfite	5.4	26.80	23.36	0.088
	None	4.6	25.60	23.56	0.090
16	Molasses	4.2	25.60	27.24	0.107
	Sodium bisulfite	5.4	26.00	20.80	0.081
	None	4.6	27.80	22.84	0.082
32	Molasses	4.2	25.60	23.84	0.094
	Sodium bisulfite	4.9	25.20	21.72	0.087
	None	4.5	24.20	24.52	0.103
64	Molasses	4.2	27.60	25.04	0.092
	Sodium bisulfite	4.5	25.20	22.96	0.090
	None	4.7	23.20	21.28	0.092
128	Molasses	4.5	23.20	22.80	0.099
	Sodium bisulfite	4.5	24.40	21.04	0.087
Control—dehydrated alfalfa			27.00	24.92	0.094

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SOLIDS-NOT-FAT IN MILK SYMPOSIUM

This symposium was sponsored by the A.D.S.A. Dairy Cattle Breeding Committee, a joint committee of the Production and Extension Sections. Its purpose was to summarize research on the solids-not-fat and other components in milk, and to exchange ideas.

The discussion that followed the presentation of papers indicated a need for an extended study on farm herds throughout the country. To bring this about, the Production and Extension Sections requested that the A.D.S.A. Dairy Cattle Breeding Committee appoint a committee to develop a cooperative research program among the various experiment stations. The committee appointed was: D. MADDEN, Michigan State Univ., East Lansing, *Chairman;* J. E. LEGATES, North Carolina State College, Raleigh; W. J. TYLER, Univ. of Wisconsin, Madison, and S. N. GAUNT, Univ. of Massachusetts, Amherst. The committee has contacted experiment stations throughout the country to ascertain their interest in cooperating in the project and over 20 states have indicated an interest. Details of agreements, standardization of procedures, equipment, data collection, and methods of analysis are being studied.

—S. N. GAUNT, *Chairman, A.D.S.A. Dairy Cattle Breeding Committee*

NONGENETIC FACTORS AFFECTING THE PRODUCTION OF NONFAT MILK SOLIDS BY THE BOVINE[1]

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Factors that affect the composition and total production of milk by the bovine include: breed, individuality, nutrition, locality, bioclimatological effects, management, stage of lactation, and physiological factors (age, exercise, estrus, gestation, yield, sickness, etc.). Many of these factors are inter-related and for some the effect may be small, except under exceptional conditions. The major factors affecting the production of nonfat milk solids on which there has been considerable work are breed, individuality, nutrition, bioclimatological effects, and stage of lactation. Breed and individuality are more properly thought of as genetic factors. An attempt will be made here to review briefly some of the knowledge pertaining to bioclimatological and nutritional effects; then to conclude with some studies concerning protein production and the stage of lactation.

It is important to consider, however, some concepts regarding the nonfat solids of milk. Careful judgment is necessary in the current economic switch in emphasis from butterfat to the nonfat solids. It is well known that cow's milk is a nutritionally valuable product, in that it was designed by nature to supply nourishment for the young animal. That it fulfills this function and is also advantageous for the growing and adult animal is supported by chemical analyses, which show that milk contains a favorable balance of fat, carbohydrate, and protein fortified with minerals and vitamins *(7)*. However, in the light of current knowledge, the relative importance of each of the constituents should be appreciated. Due to the availability from other sources of many of the nutrients present in milk it appears that in the future protein will be the constituent which justifies milk nutritionally and, as nutritional awareness increases, most likely it will be the component which will justify milk economically. We are living in an age where a nutritional analysis of contents is appearing even on the labels on cans of dog food. World-wide, there is a shortage of food calories as such *(18)*. However, there is a greater shortage of high biological-value proteins *(20)*. The ability of the dairy cow as a ruminant to convert low-grade roughage and nitrogen sources into high biological-value protein is perhaps its most important asset.

Lactose (milk sugar) is a source of energy; however, glucose is available much less expensively from other sources; also, there is no biochemical evidence that preformed lactose or the galactose portion of lactose is essential nutritionally even for the new-born *(7)*. The small amount of galactose required for the developing nervous tissue can be supplied amply by the Waldenase enzyme system present, which interconverts glucose and galactose *(7)*. By this same mechanism, the galactose portion of lactose is converted to glucose, where it can be utilized by the animal. It is well known that a good portion of the consumption of calcium and phosphorus of the American people is from milk. However, this in no way justifies milk,

[1] Presented at 52nd Annual Meeting of the American Dairy Science Association, Oklahoma State University, Stillwater.

since if it did, enterprising enterpreneurs would soon supply the market with more direct and less expensive sources of calcium and phosphorus than milk. Thus, caution is necessary in not putting too much weight on the total nonfat solids but in giving consideration to the relative importance of each of the constituents. Since it is believed that protein is the most important constituent, the emphasis in this discussion will be devoted to the milk proteins.

It is pertinent to point out the paradoxical situation which arises when all of the nonfat solids are considered to be of equal value. On a basis of total nonfat solids present in milk per se, a good argument can be made in favor of the Holstein breed over the breeds producing higher fat. Certainly, Holsteins produce milk containing more of the caloric content present in the nonfat solids relative to the butterfat *(11)*. However, in producing more milk with less butterfat, the Holstein also is producing more total lactose in that milk. If one is to be fair, the caloric contribution of fat plus lactose should be compared to that of protein. On this basis, it is hard to find much difference between the common breeds in their protein-producing abilities. It must be recognized, however, that large differences do exist between individual animals.

Specific factors affecting the nonfat solids milk production. *a*) Bioclimatological effects—The fact that a decrease occurs in the fat and nonfat solids composition, as well as in the total production of milk during the summer months, has been well established. However, in analyzing some of the older studies, which show large compositional decreases during the summer, it is apparent that many of them were herds in areas where there was a question of nutritional adequacy in the summer pastures or where there was a tendency for more cows to freshen at one time of the year, thereby introducing a stage-of-lactation effect. Various investigators have made excellent reviews of the literature concerning some of these aspects *(5, 12)*.

In dry years, there is a tendency for the total yield of milk to decrease, along with a decrease in the nonfat solids composition and an increase in the fat composition. In wet years, there is a tendency for both the nonfat solids and the fat percentage to decrease, with or without an increase in the pounds of milk, depending on the level of nutrition. These effects are not as important to consider today, when dairy cows are not allowed to shift for themselves on range and pasture as they used to, and when adequate nutritional feeds and supplements can be made available at all times.

The effects of bioclimatological factors on milk production have undergone considerable scrutiny in recent years, with emphasis on the programs for developing suitable cattle breeds and strains for different climates. Studies of the Missouri investigators indicate that the decrease in feed consumption at higher temperatures is apparently the major reason for decreased milk production *(3)*. The water consumption of high-producing cows decreases and of low producers increases. Such changes start in the range of from 70 to 85° F. for the common North European breeds. A rise in fat and total solids composition of the milk occurred above 80-90° F., varying somewhat with the breed and individual *(4)*. Various workers *(2, 13)* have correlated the increase in body temperature with the decrease in feed consumption and in total milk production. Studies at the more northern experiment stations have shown that the common breeds are most resistant to cold temperatures than was previously thought, and that milk production and composition do not suffer.

b) Nutritional effects—Many of the reports in the older literature describing bioclimatological effects were actually describing effects indirectly caused by the nutritive feed potential available to the lactating cow *(12, 15)*. A high-producing cow needs a smaller percentage of its feed intake for maintenance and produces relatively more milk. This milk tends to be of a lower fat composition, but contains more energy present as protein and lactose.

It is not the idea here to discuss dairy cattle nutrition. However, one can point out some principles concerning milk produced by cows, either in starvation or in overabundance of feeding. The composition of the individual protein constituents of milk in either case is probably not altered materially, although the relative composition may be. For example, whether starved or well-fed, cows produce milk containing the same amino acid composition in the α-casein; in starvation there is just less of it. Proteins fed to the cow do not appear in the milk. This is also true of the sugars, for if sugars different from those appearing in milk are fed, they do not appear in the milk. An exception to this is butterfat. If certain long-chain fatty acids are fed they may appear in the milk as part of the butterfat. The same is true of certain minerals also, but not of others.

Many of the reports in the older literature relating to effects of locality are today directly traceable to nutritional shortages. Reid *(15)* has made an excellent report on these as well as other aspects. The effects on total production of excess molybdenum, selenium, and zinc in the diet are apparent, as are the effects of deficiencies, such as iodine or cobalt. Today, through proper nutritional understanding, adjustments can be made correctly to allocate the dietary intake of these materials.

c) Stage of lactation—This is probably one of the largest factors contributing to daily and monthly variations in the nonfat solids production and protein composition of milk of the individual cow. An appreciation of it is necessary in considering the productive capacity of an individual cow during the entire lactation.

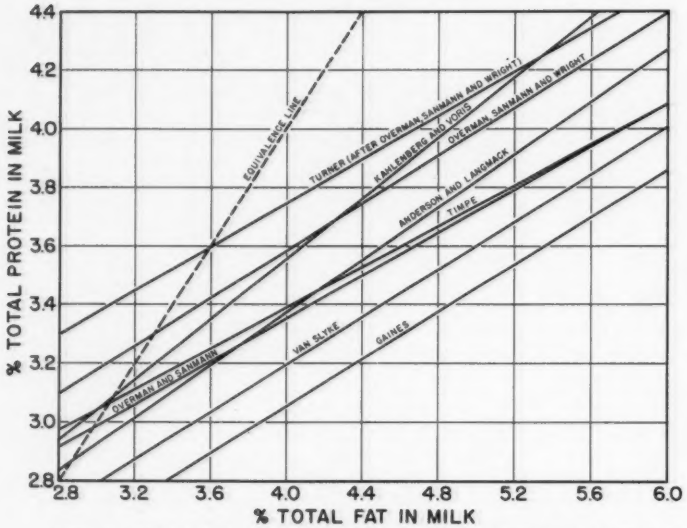

FIG. 1. Relationship between the protein and fat content of milk as found by several investigators. [Table taken partially from McDowall *(12)*, where the references not quoted in the text may be found. Some may be found also in reference *(11)*.]

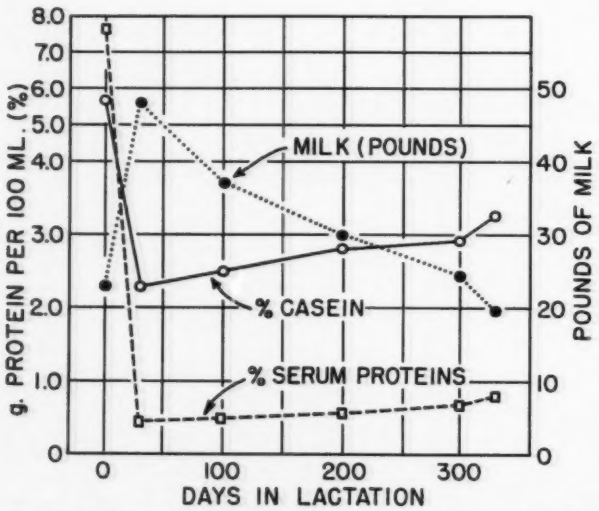

FIG. 2. Effect of stage of lactation on the total casein and whey protein composition of milk.

Large day-to-day fluctuations occur in the total butterfat yield (in per cent composition, but more so in total yield per day) *(11)*. Because the percentage of lactose is quite constant in milk *(14)*, its daily production varies with the pounds of milk produced. Total protein yield, however, tends to be more constant day to day *(1, 11)*.

Large changes occur in the ratio of protein to fat during the lactation period, varying considerably with the breed and individual *(11)*. Starting at about 2.0 at parturition, the ratio decreases to about 0.7 at the time of maximum milk yield, increases slowly to about 0.95 at 300 days, and then decreases to about 0.80 at 330 days if the cow is not dried off. Such differences point up only one of the reasons why such widely varying relationships between

the fat and protein content of milk have been found. Some of these relationships are shown (Figure 1). Analysis of these relationships found by various investigators shows that there is up to a 30% discrepancy between the highest and the lowest correlation lines. It is interesting to note that the relationship *(19)* most commonly quoted or reproduced *(5)* does not agree with the authors' data from which it was constructed *(14)*, and is the line farthest away from the others. Even though the positive correlation indicates that a high-fat milk is higher in protein also, it should be noted that it is not equivalently higher, because for each increment of more protein a greater amout of fat is present. On a caloric basis (nine and four calories per gram for fat and protein, respectively), the difference is even more striking. The reasons for such discrepancies in the relationships are apparent when breed, stage of lactation, nutritional, and other effects were not taken into proper consideration. The dangers in assuming per se that breeding for high-fat yield leads also to high-protein yield also are apparent.

During the lactation period, definite changes occur in the protein composition of milk *(11)*. The average casein and whey protein compositions of milk are high at parturition, reach a minimum at the time of maximum milk yield, and then increase to the end of lactation. This is illustrated (Figure 2). In addition to the changes in the gross protein composition of the milk, the curves for total protein production also show characteristic trends. This is true also of the individual protein components *(10, 11)*.

At this point, it is necessary to review some facts concerning the major protein components of milk. α-, β-, and γ-Casein are the major proteins of casein, and the immune globulins, α-lactalbumin, β-lactoglobulin, serum albumin, and some other minor identified and unidentified proteins comprise the whey proteins. The immune globulin fraction, which is highest in colostrum but also present in normal milk, composes essentially the classical "lactoglobulin" fraction; β-lactoglobulin is the chief component of the classical "lactalbumin" fraction. This nomenclature has undergone revision in recent years and today the terms "lactoglobulin" and "lactalbumin" are outmoded *(8)*. Instead, the names of the individual proteins comprising them, with the suitable prefixes, should be used.

These individual proteins possess different properties and arise from two different origins. Experiments conducted in this laboratory *(9)*. with C^{14} have indicated that the four proteins, α-casein, β-casein, α-lactalbumin, and β-lactoglobulin, are proteins synthesized from a free amino acid pool in the secretory cells of the mammary gland. γ-Casein, the immune globulin, and milk serum albumin apparently are proteins which enter the gland and milk preformed from the blood stream.

The production of proteins synthesized in the secretory cells of the mammary gland in most cows tends to reach a maximum within a week after parturition, then declines to the end of lactation, with a slight increase shown at the time of maximum milk yield *(10)*. In contrast, like the production of total milk, the daily yields of fat and lactose tend to reach a maximum together about a month after parturition. The relative compositions of the proteins not synthesized in the gland but coming from the blood are high at parturition, reach a minimum at the time of maximum milk yield, then start increasing to the end of the lactation period *(10)*. However, the total production of these proteins keeps decreasing through the lactation, but not quite as rapidly as the proteins synthesized in the gland.

Unfortunately, with the economic spotlight on fat for so many generations, much knowledge is lacking concerning the protein-producing abilities of the dairy cow, but we have attempted to fill in a little of that gap.

Man has made great strides in controlling his environment and that of his stock animals. Many factors important years ago are now being controlled through scientific understanding of their principles. This is especially true of many bioclimatological and nutritional factors. The elucidation of the biochemical mechanisms by which physiological and nutritional factors mediate milk production, and how they can be controlled to further man's desires, await greater understanding.

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ASSAY FOR THE SOLIDS-NOT-FAT OF COW'S MILK[1,2]

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The recently revived interest in the solids-not-fat (SNF) content of milk has been stimulated by *1*) the current surplus of fats, *2*) calorie-conscious milk consumers, and *3*) a recognition of the presence of high-quality proteins in this portion of the milk. Ultimately, these factors may bring forth a greater effort on the part of dairy cattle breeders to increase the SNF content of milk, along with a more realistic milk-pricing plan designed to recognize the real value of SNF. Such plans, in varied versions, are being employed presently in a few manufacturing areas. Despite the apparent selling points, we must recognize that such pricing schemes have little merit in a fluid milk market. It should be stated here that the present situation of surplus and subsidy is not conducive to a change in the pricing structure. However, it is conceivable that the present pattern will be altered in years to come if high-quality proteins are forced into a competitive market. In such a market we must be ready to launch into a breeding and testing prgram geared to the SNF portion of milk.

Laboratory methods for estimating the major components of the SNF fraction—moisture, proteins, lactose, and ash—are well established. Today, our primary emphasis is being directed toward the development of simple but accurate procedures suitable for use in a field-testing program, such as the present set-up found in the DHIA program. The components most likely to come under consideration for direct measurements are SNF, T.S. (moisture), and/or proteins. Methods for estimating lactose and ash are relegated to the laboratory situation.

The methods seemingly most suitable for the routine estimation of SNF components are discussed:

Specific gravity determinations. Hydrometric techniques have been investigated extensively and offer a practical approach to the estimation of the major components of milk. The most promising techniques are:

Lactometers. 1) The Quevenne lactometer, read at 60° F. in milk previously warmed to 105° F. and immediately cooled, furnishes specific gravity readings suitable for substitution in the estimation equation T.S. (%) = 0.25 L + 1.2 × F (%) to give values for individual cow's-milk samples approximating gravimetric determinations *(4)*.

2) The Watson-pattern lactometer *(23)*, designed to be read at 102° F., has received considerable attention over the past four years. In the laboratory, where water bath temperatures can be maintained at 102° F., this lactometer along with the estimation equation

$$\text{T. S. (\%)} = 1.33\text{F (\%)} + \frac{273\text{L}}{\text{L} + 1000} - 0.40$$ has proven satisfactory. A recently designed instrument, measuring only 6 in. over-all and graduated from 26 to 33 lactometer degrees, requiring but 4 oz. of sample, has been laboratory-tested *(17)*. This instrument is being "field-

[1] Presented at the Solids-not-fat Symposium, A.D.S.A., Oklahoma State University, Stillwater. June 28, 1957.

[2] Approved for publication as Journal Article 2210 from the Michigan Agricultural Experiment Station, East Lansing, Michigan.

tested" with encouraging results. Minor modifications in its design are recommended.

3) The Dutch-pattern Quevenne lactometer *(22)*, designed to read at 68° F. and measuring 8 in. over-all, requiring only 4 oz. of sample, has been employed with the estimation equation $\text{T.S.}(\%) = 1.23\text{F} + \frac{260\,(\text{L} + 1.8)}{\text{L} + 1000}$.

Recent studies made at this laboratory indicate that this lactometer and/or its estimation equation is not as satisfactory for measuring the T.S. of individual cow's milk samples as other lactometric techniques *(17)*.

Westphal balance. Specific gravity determinations are made by a balancing technique obviously too tedious for the degree of accuracy achieved *(10)*.

Subsurface hydrometer. This instrument is being developed at Washington State by Golding *(9)* and is based on the same hydrometric principle as the lactometer. A set of plastic disks of varying densities and arranged on a stainless steel rod is placed in a tube of milk and the entire assembly is centrifuged in a Babcock centrifuge. The suspended disk, marking the dividing point between the floating pack and the bottom cluster, corresponds to the density of the skimmilk portion of the milk. This value is relative to lactometer degrees and is applied in estimation equations for the T.S. or SNF values. The technique is ingenious and can be used to measure the specific gravity of the skimmilk portion. To demonstrate its practicability, the instrument should be field-tested.

Falling drop. Barbour and Hamilton *(5)* devised a technique for measuring the specific gravity of a drop of blood by comparing its falling time to the falling time of a standard salt solution in an immiscible liquid. This interesting technique, applied to milk, is being studied in our laboratory. Preliminary results are encouraging. If the method proves to be reliable, it will incorporate the advantages of speed and small sample size. A potential application exists in its use by a central testing laboratory.

All hydrometric methods are subject to the general criticism that relatively accurate density values must be substituted into estimation equations developed from mass data. The milk samples of nonaverage or abnormal individuals, not fitting such an equation, are evaluated erroneously. This weakness can be modified somewhat, and the estimation equations made more sensitive, by deriving specific estimation equations for the herd and/or area under test.

Gravimetric determination of total solids. Direct measurements of total solids, by determining the residue weight following the removal of moisture by heat, constitute the basis for the most reliable measuring techniques.

Method of the Association of Official Agricultural Chemists. This oven-drying technique requires three painstaking, analytical weighings and several hours of oven-time *(3)*. Although an accepted standard for moisture (T.S.) determinations, the method requires equipment much too elaborate and sensitive for field conditions. Large numbers of samples present a real problem unless analyzed under highly organized laboratory conditions.

The Mojonnier modification. Moisture (T.S.) determinations have been accelerated considerably by the use of a vacuum oven *(18)*. In recent years, various modifications of the vacuum oven and changes in operating procedures have made this method even faster *(15)*.

Short-cut drying and weighing techniques. a) Infra-red lamps employed as a source of heat and in combination with torsion wire balances have succeeded to a degree in making the dry residue–weight technique a field method *(6)*. Unfortunately, these techniques are for single sample determinations and have not proved successful under conditions encountered in the field.

The oxidometric method. A ceric sulfate method recently reported claims a high correlation with gravimetric determinations *(16)*. Some difficulty with the rather indefinite and unstable endpoint of the reaction mixture has been experienced. Until this tricky endpoint is mastered, the technique does not appear to have field possibilities, although the manipulations involved are but simple titrations.

Protein measurements. Many dairy people believe that the proteins of milk are its primary asset and should serve as the basis for measurement and evaluation. The standard procedures for protein determinations are laboratory techniques based on the Kjeldahl method and obviously are too involved to be used as a field method or for large numbers of samples. However, rapid and fairly simple methods for the estimation of the protein content of milk have been used with some success. A few of these methods possess possibilities as field methods.

Formol titration. The formol titration is an old friend of the protein chemist and has been adapted to the estimation of protein for many purposes. In fact, many of the dairy plants in Europe utilize the formol technique to measure the protein content of patrons' milk *(8)*. The procedure reported by Richardson *et al. (20)* at Oregon State has possibilities of becoming a satisfactory field test for the estimation of the protein content of milk. This method consists of a simple titration utilizing a standard sodium hydroxide solution to the phenolphthalein endpoint before and following the addition of a small quantity of formalin.

Titration to pH 4. The buffering capacity of milk, owing to proteins and salts, is utilized to estimate its protein content by a procedure developed at Washington State by Ashworth *et al. (1)*. The method has laboratory possibilities as a method capable of analyzing large numbers of samples, but does not appear to be suitable for field testing because of the equipment and techniques involved.

Dye binding. A rapid, simple procedure for measuring milk proteins has been reported by Ashworth and Seals at Washington State *(2)*. The test is based upon the ability of the disulfonic acid dye, Orange *G*, to bind with the basic groups of the protein molecule. The test has given satisfactory results and appears to have the potentiality of becoming a method suitable for the routine laboratory analysis of normal milk samples.

Protein components. Methods available for estimating the protein complement of milk are laboratory procedures represented by techniques such as electrophoresis, chromatography, and solubility. Because of the complexity and time requirements of these techniques, it is doubtful that large-scale evaluations could be undertaken *(21)*.

Prediction equations. Derivative equations, obtained from mass data, offer a practical means of estimating indirectly the SNF or T.S. content of milk from fat, protein, or other highly correlated component relationship. There are inherent errors associated with this technique which must be recognized. Most prominent among these is that the prediction equations represent mean relationships; therefore, they do not predict accurately at the extremes *(11, 12, 13, 14, 19)*. Whether this constitutes a practical objection is debatable.

Other methods. Additional methods for the determination of milk solids, as studied by Davey and Patton *(7)*, include chemical oxygen demand, hydrolysis of acetyl chloride in the presence of pyridine by water, Karl Fischer reagent, modified drying procedures, and others.

Conclusions

The small Watson-pattern lactometer offers a method sufficiently accurate and simple to use in large-scale testing programs, either in a laboratory or in field application.

The formol titration appears to be best suited as a rapid and simple method for the estimation of the protein content of milk under field conditions.

A recently developed technique utilizing the protein-binding capacity of Orange *G* might possibly offer a practical method for measuring the protein of milk at the laboratory level.

More sensitive estimation equations for T.S. and SNF from lactometer readings, and corresponding prediction equations for estimating highly correlated components, can be derived from mass data obtained under local conditions.

The direct determination of T.S. of SNF by sensitive gravimetric techniques remains in the realm of laboratory procedure.

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GENETIC ASPECTS OF SOLIDS-NOT-FAT AND ITS COMPONENTS[1]

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The evidence for genetic variation in the composition of milk may be divided into three sources: (a) Breed differences, (b) sire differances, and (c) individual differences.

Breed differences. The fact that differences exist among breeds in the composition of milk has been known for some time. A comprehensive study of this problem was made by Overman and coworkers *(11, 12)* and a table prepared by Turner *(19)* from these data has received wide publication in many of our dairy science textbooks. Figures from this table are given (Table 1).

TABLE 1

Breed differences in composition of milk

Breed	Component of milk					
	Fat	Protein	Lactose	Ash	T.S.	S.N.F.
Ayrshire	4.00	3.53	4.67	0.68	12.90	8.90
Swiss	4.01	3.61	5.04	0.73	13.41	9.40
Guernsey	4.95	3.91	4.93	0.74	14.61	9.66
Holstein	3.40	3.32	4.87	0.68	12.26	8.86
Jersey	5.37	3.92	4.93	0.71	14.91	9.54

Bailey *(1)*, in a recent review article, has pointed out that there appear to be strain or herd differences in milk composition. This seems to be important, because research by many workers has indicated that the figures in Table 1 may not be indicative of each breed's average. For example, Overman *(10)*, in later studies of the milk of Brown Swiss cows in 39 herds, reported the milk composition to be: 3.97% B.F.; 9.16% S.N.F.; 3.52% protein; 4.90% lactose; and 0.74% ash.

Data from Combs, Reaves, and Graf *(3)* showed that the S.N.F. of Guernseys is 9.18%, which is considerably lower than Overman's figure.

In the Holstein-Friesian breed many investigators *(2, 3, 7, 14, 21)* have reported the average S.N.F. per cent to be between 8.46 and 8.60; whereas, others *(2, 3, 7, 14, 17)* have found the average S.N.F. per cent for Jersey cows to be from 9.32 to 9.53. Robertson's *(15)* figures for composition of milk of Ayrshire cows in Scotland were similar to Overman's values.

Recently, Rolleri *et al. (16)* have studied the differences between the five dairy breeds in the components of protein. They reported that the milk from Holstein cows has less total alpha- and beta-, but more gamma-casein, than the milk of other breeds of dairy cattle. When the proportions of the casein were considered, Holstein milk had less beta- but more gamma-casein, whereas the Ayrshire milk had more beta-casein than the other breeds.

The evidence from breed data certainly is indicative of a genetic difference in composition of the milk of dairy cattle.

Sire differences. Variation among sires in the average composition of their daughters' milk for S.N.F. has been reported by several workers *(8, 9, 14, 21)*. The interpretation of such data needs to be made with caution when such data extend over a long period of time, since environmental factors such as age, climate, and feed may be responsible for a sizable portion of the sire differences. However, recent information from Holland and Great Britain *(13, 15)* indicates that daughters of sires used in A.I. centers, and sires whose daughters are tested in special progeny test stations, exhibit differences in protein and S.N.F. content of their milk.

Individual differences. The evidence for heritable differences in milk composition between individual cows has received only scant consideration from research workers. The bulk of the evidence stems from studies of identical twins in Sweden and New Zealand *(4, 5, 6, 22)*. The heritability figures derived from research with identical twins are given (Table 2). Such figures include not only the additive gene effects but also dominance and epistatic effects. Robertson believes that these figures are biased upwards and include an environmental biats.

The use of dam-daughter relationships to estimate heritability (in the narrow sense, because

[1] Paper No. 682, Department of Genetics.

TABLE 2

Heritability estimates (twin data)

Source	Component of milk	Heritability
	(%)	
Sweden *(5)*	Fat	0.87
	Protein	0.88
	Lactose	0.62
New Zealand *(4)*	Fat	0.95
	Casein	0.94

all the dominance effects and most of the epistatic effects will be missing) of milk composition has been reported by Robertson *(15)*. The data used in his study were age-corrected, single-lactation records of 300 dam-daughter pairs of the Ayrshire breed. The estimates of heritability are given (Table 3). Also in this

TABLE 3

Heritability estimates (dam-daughter data)

Source	Component of milk	Heritability
	(%)	
Scotland *(15)*	Fat	0.32
	Protein	0.48
	Casein	0.58
	Lactose	0.36
	Ash	0.50
	S.N.F.	0.53
Netherlands *(13)*	Protein	
	Lactose	0.5 to 0.7
	S.N.F.	

table are presented some values computed by Politiek *(13)* in an analysis of Friesian data in the Netherlands. The two reports show that heritabilities of individual components of milk are moderately high.

There have been many studies of the association between some of the constituents of milk *(12, 18)*. However, in this report only the data presented by Robertson *(15)* are given (in the right-hand portion of Table 4). The lower portion of Table 4 shows the genetic correlations between constituents, also reported by Robertson. The genetic correlations between casein and both fat and lactose were positive and significant. The genetic correlation measures the association between two characters, because the same genes have effects on both characters. The phenotypic correlation contains this genetic component and also a contribution from environmental factors affecting both traits. The genetic correlation is useful in predicting change in one character while selecting for another. Because of the small number of pairs involved in this study, the standard error of the genetic correlation is large.

Summary

Evidence has been presented which indicates that part of the variation in composition of milk is genetic and that selection for S.N.F. or its basic constituents should be effective in changing the composition of milk of our future dairy-cow population. However, additional data are needed in order to obtain more precise estimates of the genetic parameters, i.e., heritabilities and genetic correlations.

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TABLE 4

Phenotypic and genetic correlations (within herds)

	Yield	Fat	S.N.F.	Crude protein	Casein	Lactose	Ash	
Yield		−0.14	−0.18	−0.26	−0.20	0.08	−0.05	Phenotypic correlations
Fat	−0.01		0.40	0.42	0.43	0.11	−0.03	
S.N.F.	−0.02	0.46		0.81	0.87	0.50	0.12	
Crude protein	0.22	0.48	0.94		0.93	−0.01	0.10	
Casein	0.05	0.56	0.82	0.96		0.17	0.00	
Lactose	−0.16	0.37	0.67	0.41	0.41		−0.28	
Ash	−0.10	−0.42	−0.42	−0.10	−0.24	−0.86		
	Genetic correlations							

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PROPOSED PLAN FOR COOPERATIVE STUDY OF THE INHERITANCE OF MILK COMPOSITION

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Experiments concerning the inheritance of economic factors not previously studied extensively involve essentially the same pattern of procedure; namely, environmental factors which affect characters, adjustments which should be made, and the genetic parameters which can be estimated.

I. General considerations

A. *Environmental factors affecting milk composition:*

1. Age at calving
2. Stage and length of lactation
3. Month of calving
4. Breed
5. Calving interval, days dry, gestation period
6. Other factors: geographic and managemental effects, disease and other unusual circumstances.

B. *Adjustments to be made.* Using methods of least squares, one can measure and adjust for the effects found to be significant. These adjusted data then can be used to estimate the following genetic parameters:

Repeatability indicates the reliability of a single record as an indication of breeding value. If it is of sufficient magnitude, cows could be sampled for a single lactation or for periods during a lactation.

Heritability represents the relative magnitude of the genetic variability and, depending on its magnitude, indicates the selecting procedures which should be followed to achieve maximum genetic progress.

Genetic and phenotypic correlations of the milk components with each other and with other production characters to provide information required to establish optimum schemes for simultaneous selection of several traits.

II. Recommended procedures

A. Collecting much data for several years will be necessary to estimate adequately the desired statistics. Herds could be selected which would give a high frequency of daughter-dam pairs or which would maintain daughters of AB sires.

It is recommended that cattle in various geographic areas, and in various herd levels within these areas, be sampled routinely by the DHIA Supervisor. At least two locations each should be selected, in the East, South, Midwest, and West, to sample cows in various production levels.

Sampling 3,000 animals in each breed for from 3 to 5 yr. should result in sufficient records to estimate repeatabilities and heritabilities. These figures must be arbitrary, based on statistics which vary among herds and probably are, at best, only incompletely known.

B. Methods of testing will depend on the available testing procedures and on the importance of the following components:

Solids-not-fat is most important economically and can be measured accurately in the field by the DHIA Supervisor, using the small Watson Lactometer. Therefore, maximum effort should be placed on solids-not-fat.

Protein, total nitrogen, protein components, etc. are important to know, both economically and physiologically. These cannot be measured accurately in the field, and they involve tedious and expensive laboratory procedures. Some of these detailed data should be obtained on large numbers of cows, but they will be expensive and difficult to obtain.

Lactose and minerals are relatively unimportant economically, but have sufficient physiological importance to warrant laboratory consideration.

C. Testing equipment and methods should be investigated continuously, in an attempt to estimate milk components more accurately and economically.

The suggested procedures indicate that solids-not-fat should be measured extensively, that protein measurements should be made (but will be limited at present) on as many animals as possible, and that limited consideration should be given to lactose and ash.

OUR INDUSTRY TODAY

THYROPROTEIN FOR DAIRY CATTLE

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Thyroprotein is a hormone-containing product which produces an increase in milk production when fed to dairy cattle. The question of whether the increase in milk production is sustained, along with other questions of feeding the product, is discussed in the following presentation. The product has been available commercially since about 1945; its sale has varied considerably from time to time and from locality to locality and reached a minimum about 1954. Recent sales effort has increased the use of the product in some areas.

The product is manufactured by the iodination of casein under certain specified conditions. The active agent in the product is the hormone, thyroxine, the same hormone that is secreted by the thyroid gland. The thyroprotein is usually mixed with some feedstuff and sold as a supplement to be fed in addition to the usual ration.

The most recent technical review of the various aspects of feeding thyroprotein to dairy cattle was published by the National Research Council *(5)*. The review of the English and American work was concerned largely with the effects of feeding thyroprotein over a greater part of the lactation period. It was pointed out that the feeding of thyroprotein results in an average temporary increase in milk production of from 15 to 20% above control animals, but that the total milk production for the lactation was not increased when the same level of energy intake was maintained. The following statement in the summary of the review is given, "The available data suggest no definite economic advantage of feeding thyroprotein to dairy cows under most farm conditions. It appears more desirable for the dairyman who raises his own herd replacements to refrain from using thyroprotein, especially if the calf mortality observations are confirmed. It may, however, be advantageous to use thyroprotein in well-fed commercial herds in which maximum production per cow is emphasized, and in herds during periods of short milk supply where this is permitted by law and where other regulations do not prohibit its use. It probably should not be fed for periods longer than 90 to 120 days and not during the first 50 days of lactation or in the terminal part of lactation."

The question of interpretation of response following thyroprotein feeding needs some discussion, especially in relation to energy intake of control and thyroprotein-fed animals. Two procedures have usually been followed in both short- and long-term experiments. *(1)* The control group is fed according to requirements, whereas the thyroprotein group is fed either the requirements plus the nutrients in 3 to 5 lb. of concentrate or at a certain given per cent above requirements. As milk production increases, owing to thyroprotein stimulation, grain is proportionately increased. *(2)* Both groups are fed according to requirements for maintenance and milk production or a certain per cent above requirements.

It was found in the early results reported from Beltsville and discussed in the Technical Review *(3)* that if No. 1 was followed, in which thyroprotein was fed 50 days post-partum for the remainder of the lactation period, milk production for the thyroprotein-fed animals exceeded the control animals for the lactation period. This effect is, of course, owing both to extra feed and to thyroprotein. When No. 2 was followed, milk production for the thyroprotein-fed animals was above that of the controls for a period of from 60 to 120 days, after which the level fell below that of the controls, so that there was little difference in production for the lactation period, regardless of whether both groups were fed at requirements or at 25% above requirements. This measured the effect of thyroprotein per se. Therefore, it was concluded from the long-term experiments that unless the dairy farmer is interested in producing extra milk for a certain period for the market it would appear very doubtful that it would pay to feed thyroprotein. He probably could obtain about the same production for the lactation period by feeding extra grain without thyroprotein.

Thus, in measuring effects, No. 1 is due to extra feed and thyroprotein, whereas No. 2 is due to thyroprotein. It is felt that the latter procedure provides the only fair basis to assess the true value of thyroprotein. Only when a distinction is made between extra feed and thyroprotein effect can the true effect of thyroprotein feeding be assessed. This is true for both short- and long-term feeding of thyroprotein. This consideration usually has not been made clear in the popular press, in articles which discussed thyroprotein feeding, where no account of the extra feed intake has been considered and with the inference that the results are owing solely to the feeding of thyroprotein.

Although control cows usually will not consume as much feed as thyroprotein-fed cows, they will consume more than the Morrison

standard requirements, and many individual cows, if given the opportunity, will approach the amount consumed by thyroprotein-fed cows.

In unpublished data from Beltsville, five sets of identical twins were used, in which one animal of each set was fed thyroprotein at the rate of 1.2 g. per 100 lb. of body weight, beginning 50 days post-partum for the remainder of the lactation. Extra feed was offered simultaneously, equal to 125% of Morrison's maximum requirement. The other member of each set served as a control, being fed no thyroprotein but fed at 125% of requirement. The controls produced 9,734 lb. of FCM for the lactation, whereas the thyroprotein-fed animals produced 9,245 lb. Actual consumption of TDN for the entire lactation as compared to Morrison's requirement was 106% in both groups. One set of triplets was included in the experiment; one of the triplets was fed at Morrison's standard and produced 9,110 lb. of FCM; another of this triplet set was fed at 125% of Morrison's standard and produced 11,195 lb.; the thyroprotein-fed triplet was fed at 125% and produced 9,988 lb. of FCM. Actual feed consumption in comparison to Morrison's standard was 93, 111, and 109%, respectively. These results confirm the early long-term feeding experiments conducted at Beltsville and again point up the importance of feed or energy intake in the interpretation of results.

Since the reporting of the long-term thyroprotein-feeding results from Beltsville and publication of the Technical Review *(5)*, attention has been turned toward short-term feeding of thyroprotein, for periods of from 60 to 120 days. When thyroprotein is fed for short periods, questions concerning the yield of milk and economic returns have been in recent controversy as affected by, *1*) the length of the period of stimulation of milk production in relation to production for the lactation; *2*) the level of feed intake; and *3*) the method of withdrawal of thyroprotein from the ration. Results to answer some of these questions are available, although there is need for rationalization of disputed points in the light of the previous discussion.

Experiments on short-term feeding of thyroprotein to obtain information on milk production and economic return have been conducted at Beltsville *(6, 7)* and at the University of Tennessee *(3, 4)*. In the experiments at Beltsville *(6, 7)*, a total of 38 controls and 81 cows fed thyroprotein at the rate of 15 g. per day for 60 days have been used at four different locations under varying conditions. Accurate feed consumption data, including both concentrates and forage, milk production, and live weight records, were obtained. Data on the pre-experimental and post-experimental periods also were collected. A typical result is shown (Figure 1).

Again, it was shown that when thyroprotein was fed, milk production was increased. How-

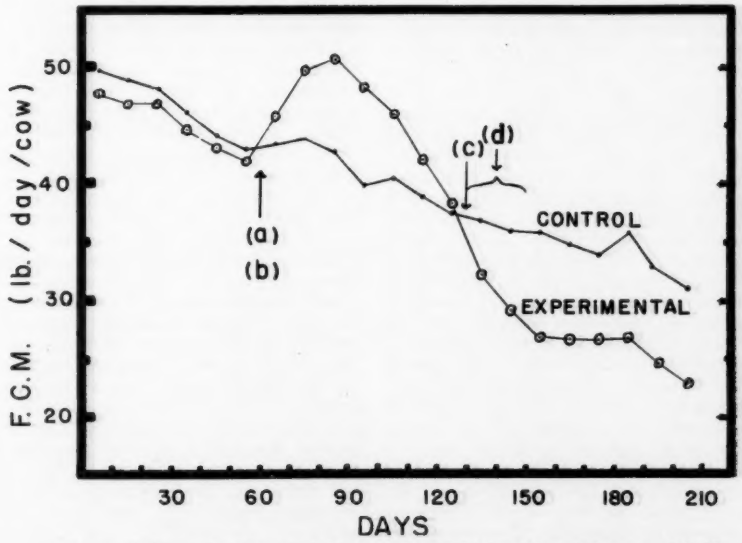

FIG. 1. FCM production of eight control and 24 experimental cows. (a) Extra grain fed to controls, (b) thyroprotein and extra grain fed to experimental cows, (c) extra grain removed from controls, (d) extra grain and thyroprotein removed from experimental cows during this period. (Data adapted from J. Dairy Sci., 40: 128. 1957.)

ever, when thyroprotein was removed from the ration, milk production decreased below the calculated expected level or the level of control animals. Calculations of monetary returns showed an advantage during the period when thyroprotein was fed. However, when the post-experimental period also was considered, much of the advantage disappeared and was dependent upon the extent of decrease in milk production which followed withdrawal of thyroprotein. Gradual withdrawal was tried and whereas milk production decreased more gradually, the final level reached was the same as when the thyroprotein was removed abruptly.

Investigators at the University of Tennessee *(3, 4)* reported that the gradual withdrawal of thyroprotein following a 100-day feeding period resulted in milk production decreasing only to the expected level of the lactation curve or to that of the controls. It would appear on the surface that the results obtained at the University of Tennessee *(3, 4)* and at Beltsville *(6, 7)* were in conflict. However a study of the data shows that the results can be rationalized in the light of the previous discussion. It is believed that the differences are due to the level of feeding of the controls in relation to the thyroprotein-fed cows during the period of thyroprotein feeding. In the Tennessee experiments, the control cows received 19.5 lb. of TDN per day, whereas the thyroprotein-fed groups received 22.2 lb. of TDN per day, or a difference of 2.7 lb. during the 100-day feeding period.

In the Beltsville short-term experiments, with one exception, the control and thyroprotein-fed groups were generally equal in their consumption of TDN, with the greatest difference being about 1.5 lb. per day. Where extra grain was added there was some concomitant decrease in hay intake which tended to equalize TDN intake. Thus, in most cases the Beltsville results actually measured the difference due to thyroprotein per se and not that due both to feed and to thyroprotein. It is believed that the results at Tennessee, in which the thyroprotein-fed animals did not decrease in yield below the controls, are not due to the gradual withdrawal of thyroprotein but to the level of energy intake. In this interpretation, there is really no point of disagreement between the results obtained at the University of Tennessee and those at Beltsville. However, it should be pointed out that the cows at the University of Tennessee were fed thyroprotein for 100 days, compared to 60-90 days at Beltsville.

In the work of Blaxter *(1)* in respect to withdrawal experiments, close reading of the text and a study of the data reveal agreement with the concept at Beltsville. He states that gradual withdrawal reduces the rate of decrease in milk production. Examination of his data where feed intake was equalized reveals that the final level of milk production was not affected, whether the withdrawal was sudden or gradual.

The effects of feeding thyroprotein on subsequent milk production and viability of calves have been in controversy. In the earlier long-term experiments conducted at Beltsville, evidence of a decrease in production levels and poor viability of calves during successive lactations was reported. No such evidence has been obtained in short-term feeding experiments. However, a word of caution should be expressed where thyroprotein is fed during the latter part of the lactation, in terms of the possible effects noted in the long-term feeding experiments. It has been noted that when thyroprotein is withdrawn from the ration, the thyroxine-secretion rate returns to normal in a period of from 3 to 4 wk. It has been stated that because thyroxine secretion returns to normal there should be no effect on the subsequent lactation or on the viability of calves born during subsequent pregnancies. Obviously, the fact that the thyroxine-secretion rate had ample time (90 days) to return to normal in the earlier long-term experiments did not prevent the effects previously noted. The effects must, therefore, be owing to some other disturbance in metabolism which was not corrected by a resumption of the normal thyroxine-secretion rate.

In this connection, it is of interest to note the results of an experiment reported in a paper of Hancock, Brumby, and Turner *(2)*, in which six identical-twin heifers were injected daily for the first 6 mo. of gestation with L-thyroxine at the rate of 0.4 mg/100 lb. of body weight. During the succeeding 3 mo. of gestation, the previously injected heifers largely caught up with their control twins in body weight and apparently regained their normal thyroxine-secretion rate. Despite the fact that both groups were treated similarly after the thyroxine injections, the heifers which had been previously injected with the thyroxine produced less milk, 3,932 to 5,051 lb. of FCM, and had shorter lactations than the control twins in their first lactation. This and other evidence reported by English workers indicates that thyroprotein feeding may cause shortened lactations.

The question of whether to feed thyroprotein for short periods is largely one of economics, considering the price of milk and the price of feed. If the farmer is establishing a base period in the market, and there is considerable advantage in the class and price of milk, it may pay him to feed thyroprotein to selected cows during the base period and sacrifice milk production later, provided the price of grain is not excessive. The feeding of extra grain without thyroprotein will usually not immediately stimulate the cows to extra milk production but will promote greater persistency. If there is no advantage to immediate stimulation for the base period, there would be no advantage on the total lactation period to feeding thyroprotein, even though the milk : grain price ratio was advantageous.

Thyroprotein should not be fed to herds when poor management exists and when the herd is underfed. Under any given situation, it should be fed only to those herds which are well-fed and well-managed. Under such a situation, the heavy feeding of grain would produce as much milk for the lactation as would feeding extra grain and thyroprotein.

Efficiency of feed utilization is increased during the short-term periods of thyroprotein feeding. However, if the post-experimental period also is considered, then there is no advantage in efficiency of feed utilization.

Some evidence indicates that thyroprotein should not be fed during the hot summer months, because of the combination of heat stress and the hyperthyroid state. Of course, the effect would be more serious in the southern than in the northern states.

Most of the evidence indicates that thyroprotein should not be fed to first-lactation animals. The greatest response has been obtained during the third and succeeding lactations. Thyroprotein should be fed only during the declining lactation phase; preferably not during the late stages of the lactation period.

The feeding of thyroprotein causes considerable loss in live weight. This effect is probably due to two factors; namely, actual loss of body fat due to the hyperthyroid state and a change in body composition and/or fill. Weight loss from the latter causes is regained readily when thyroprotein is removed from the ration; whereas, that owing to body fat loss must be compensated for by extra feed.

In conclusion, it can be stated that the feeding of thyroprotein for short periods to well-fed and well-managed cows, under special market situations, may prove to be profitable, but that the feeding of extra grain without thyroprotein will produce as much milk for the lactation as extra grain and thyroprotein. The feeding of thyroprotein by the general dairy farmer should not be advised.

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PROBLEMS IN THE ITALIAN SOFT-CHEESE INDUSTRY[1]

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Originating centuries ago in southern Italy were two well-known soft cheeses, Mozzarella and Ricotta. These fit together well in the cheese-manufacturing scheme because one cheese results from the side product of the other.

Traditionally, Ricotta, or recooked cheese, is made from the whey of Mozzarella and other "pasta-filata" types. To this whey a small amount of skimmilk is often added to improve the final texture. A joint application of acid and high heat results in a protein precipitate which is removed as cheese.

Few appreciate the world-wide importance of the "pasta-filata" principle of cheese-making. In this system the texture and grain of the cheese are achieved through a skillful stretching of curd in hot water. Provolone, Caccicavallo, and other cheeses owe their characteristics to this principle. Yet, it is a soft, nonaged cheese, the Mozzarella, which is the foundation for all pasta-filata type cheeses. Knowledge of the manufacture of Mozzarella provides a good start on knowledge of these other cheeses.

It would be interesting to speculate on the circumstances leading to the origin of Mozzarella and Ricotta, one of which was described by Varro in 37 B.C. The stretching of the curd in the former, and the flotation principle of curd in the latter, antedating that of Ivory soap, reflected certain signs of ingenuity on the part of the innovators.

Ricotta cheese in the United States. New York City is the mecca for Ricotta, especially at

[1] Presented at Research Conference at Beltsville, Maryland, October 30, 1957, held by the Eastern Utilization Research and Development Division, USDA, Philadelphia, Pa.

Easter-time, but other cities are becoming important consumer centers. Ricotta is used directly in salads, in ravioli, in cheese cake, and as cream-whipped fillings for desserts. It can be consumed also just as one eats cottage cheese. Good quality Ricotta has a soft, fragile texture and a sweet, walnut flavor.

In the East, for many years cheeses from whole milk and from part skim, usually 2% fat, have become much more popular than the product from whey. The following classification is in general use:

Ricotta—cheese from whole milk
Part-skim Ricotta
Ricotone—cheese from whey
Dry Ricotta.

In areas other than the East, relatively little cheese is made from whole or part skimmilk and the term Ricotta usually denotes cheese obtained from whey.

Principles of Ricotta cheese manufacture. Whole milk is pumped into large stainless steel kettles and enough lactic acid starter is added to adjust the titratable acidity to 0.29-0.31%. It requires from about 20 to 30 min. to heat the milk to the critical temperature level for precipitation, 175-180° F. Direct steam injection is used for the initial heating.

The first sign of a precipitate is observed by the operator as fine flocculent material, on the flat surface of a spatula which is moved constantly through the heating milk. At this point the direct steam injector is removed and heating is continued slowly by jacketed steam under 5-10 p.s.i. During this period, the curd collects at the top of the kettle, with the operator making simple smooth, sweeping movements of the curd with his spatula from the surface edge to the center. After a brief quiescent period, the curd is scooped off into perforated metal containers. These containers are cooled in running water so that the cheese attains a temperature of 40° F. as rapidly as possible before shipment to market.

Salt and ice cream stabilizer also are normal additions to cheese milk intended for Ricotta. Salt highlights the flavor and perhaps contributes to a more complete coagulation. The ice cream stabilizer prevents heavy accumulation of foam, so that the cheese-maker can detect more quickly the first signs of protein flocculation.

Though starters are the most widely used means of introducing an acid to the milk, acetic acid, tartaric acid, and phosphoric acids also are employed. Along certain Mediterranean Sea areas, sea water is often used in the manufacture of a cheese similar to Ricotta. The heavy salt concentration evidently results in a salting out of the protein.

Problems of precipitation. Difficulties are common in the coagulation of proteins of whole milk and skimmilk Ricotta. An almost simultaneous precipitation of casein, lactoalbumin, and lactoglobulin is required. At times, conditions are not entirely suitable and a second precipitation must follow, to recover fully the cheese curd. The color of the whey, clear green or milky, is a good indicator of the efficiency of the initial precipitation.

In at least two periods of the year, when cows go out to pasture in the spring, and upon their return to the stables in the fall, pronounced difficulties in effecting complete precipitation are encountered. More starter and more heat are necessary. The relative proportion of proteins in the milk and shifts in salt equilibria, so critical to evaporated milk stability, must exert an influence, but in this instance the problem is stability and not instability.

If the color of the whey in the kettle after initial precipitation is milky, sufficient citric acid is added, with brief heating, to induce a secondary coagulation. The resulting cheese is coarse and rough and is blended in with the better quality first cheese.

The turtle of the Ricotta cheese family. It is a curious sight in the manufacture of Ricotta cheese to see the mass of curd rise to the top of the kettle in an almost simultaneous movement. Why don't these curd particles settle to the bottom like most good precipitates? Occluded air between and in the curd particles must explain this action resulting in a density of the curd mass slightly lower than the liquid in which it exists. At best, this is a delicate equilibrium.

A related cheese exists, however, which made in the same manner and from the same fresh milk, will sink to the bottom of the kettle where it stays. Called Impastata, it is utilized by bakers because it makes a finer whipped pastry filling. It differs from true Ricotta by being drier and having a higher lactose concentration. The acidity of the milk through starter addition is the contributory cause for its sinking. Milk with an acidity adjusted to more than 0.35% is most likely to behave in this manner, perhaps because the curds become tougher, contracting and forcing enough air to upset the delicate equilibrium determining the direction of the cheese's movement.

Technological advances. Unfortunately, no real improvements in manufacturing methods have evolved for Ricotta cheese. This is attributable mostly to the philosophy of the Italian cheese industry, which has resisted most suggestions for change, fearing inferior cheese and higher expense.

Continuous centrifugation based on principles which apply to the new continuous manufacture of Baker's, Neufchatel, and cream cheese has been suggested. Such a process, though warranting study, apparently has not yet been tested by Italian cheese manufacturers.

Mozzarella cheese in the United States. Approximately 24 million lb. of soft Italian cheese are produced annually in New York State, much of it Mozzarella. Its increased popularity

parallels that of the well-known Italian specialty, pizza pie. Mozzarella cheese is consumed in its natural state, in the preparation of lasagne and in veal cutlet alla Parmagiana.

Presently, some confusion exists as to the nature of true Mozzarella cheese. When made in the East, the cheese generally is white, with a soft texture and a high moisture, milky consistency, and a bland flavor. When made in the Midwest, the cheese generally is yellowish, drier, and frequently displays a slight Provolone flavor. The latter often is referred to as Mozzarella-Provolone or Pizza cheese. Eastern whole-milk cheese contains about 50% moisture and 19% fat, whereas Midwestern cheese averages 46% moisture and 23% fat.

Methods of manufacture. Mozzarella cheese produced in the East is made from pasteurized milk to which lactic acid or enterococci starters are introduced with or without vinegar. Rennet is added, to form a curd within 30 min.

The curd cut with cheese knives is not cooked but remains in the whey for a short period of time, after which the whey is removed completely. The curd bed is cut up into small blocks, washed with cold water, and bundled into cloth bags. After storage at 40° F. for 24 hr., these bundles may be shipped under ice to small heat-processing centers in distant cities. Here they are brought out to a warm room to reactivate the acid fermentation so that the pH will drop to about 5.3. At this point the curd is stretched in hot water, molded, and brine-salted.

In the Midwest, a high acid-producing starter, *L. bulgaricus,* is added to the pasteurized milk. Rennet paste or rennet is used to form a curd. The curd cut with cheese knives is cooked to 117° F. Then the whey is removed and the curds are matted as large patties. At an acidity of about 0.35%, reached in a few hours, the patties are milled into long strips and placed in hot water, where kneading takes place. The hot plastic curd mass is shaped into various forms and salt-brined.

The Eastern manufacturers, as a hedge against competition, now make some of this cheese also; whereas, the Midwestern manufacturer does likewise with the high-moisture Mozzarella. The high-moisture product upon shipment over long distances to Eastern markets has displayed difficulty in maintaining peak quality.

Chemistry of Mozzarella cheese. Mozzarella cheese owes its characteristics mainly to the action of lactic acid on dicalcium paracaseinate. At a pH between 5.2-5.4, much of this compound becomes converted to monocalcium paracaseinate, which provides the strings and the sheen of the cheese. Above pH 5.4, generally the curd will not stretch; below pH 5.2, the curd will stretch but excessive losses in fat result and the cheese becomes too tough.

Some technical considerations. The soft Italian-cheese industry was forced into using pasteurized milk. Now, generally, it is agreed that pasteurized milk makes a better cheese. Many within the industry have considered other technical changes.

Bleaching of the yellowish color of cheese milk with benzyl peroxide has been attempted experimentally. The results are not all satisfactory, because the stretching qualities of the curd are reported to be adversely affected. Similarly, homogenization of milk leads to a whiter appearance, but again the curd-stretching properties are adversely affected.

Another technical development and generally accepted practice is in the freezing of Mozzarella curd, generally after it has been acid-ripened. Frozen in periods of surplus, the curd is heat-processed to a finished product when milk supplies are low. The product quality is unimpaired.

A processed Mozzarella cheese is being considered as a related type. It is more difficult to process than cheese of the Swiss or Cheddar type, but it is not impossible. Because of its good slicing properties and long-keeping qualities, processed Mozzarella certainly deserves some attention.

SUMMARY

The Italian-cheese industry is faced with many problems, but two of immediate urgency are concerned with poor sanitary quality and a lack of state and national standards of identity.

Both Ricotta and Mozzarella cheese often are heavily contaminated with mold, yeast, and coliforms. This is particularly significant with Ricotta, because supposedly it leaves the hot kettle in an almost sterilized condition. Yet, when Ricotta cheese is packaged in plastic consumer cups, dramatic evidence frequently is displayed of poor sanitary quality through exploding caps and obnoxious flavors.

Obviously, the water which comes in contact with the cheese during slow cooling might be a source of the high counts. Designs for quick-cooling air-tunnels, replacing the water, are still on the drawing-boards and may remain there indefinitely because of estimated high cost of construction. Not to be overlooked is the fact that a lack of good cleansing and sterilizing techniques in these cheese plants may be a more important contributory factor.

In the packaging of Mozzarella cheese, mold growth may spoil the product because of the high initial count and because this cheese has no natural mold inhibitors such as propionic acid. Vacuum packaging has helped to reduce the incidence of mold in packaged cheese, but with sliced Mozzarella a high vacuum of 29 in. must be maintained for effective control.

The decision of the Federal Food and Drug Administration not to promulgate standards of identity for Ricotta and Mozzarella cheese at this time has been received with some misgivings. Standards are urgently needed for the elimination of the chaos apparent in certain segments of this industry. Manufacturers would do well

to sit down together and work out on a nation-wide scale the general basis for satisfactory standards of identity.

In the present confused state of the market, much cheese labeled and sold as Mozzarella bears little or no relationship to this cheese, whether of Eastern or Midwestern origin. Resembling poor Muenster, or green Cheddar, the entry of such cheese creates a very disturbing influence on the Italian soft-cheese industry and on many consumers.

The Italian soft-cheese industry is growing at a rapid rate. To keep it healthy, new ideas are of paramount importance. Now that many cheese manufacturers are becoming receptive to technological advances, a wonderful opportunity exists for progress.

NEW DEVELOPMENTS IN THE PROPAGATION OF STARTERS[1]

M. W. Hales
Chr. Hansen's Laboratory, Inc., Milwaukee, Wisconsin

Use of More than One Culture for Making Cheese Starter

For the past couple of years, there has been an increasing number of successful cheesemakers who have regularly used from two to four lactic cultures for developing their daily needs of bulk starter. This is in direct contrast to the general practice reported in foreign literature, where selected single-strain lactics have been in use for a number of years. Why, then, are many in our cheese industry using the more complex multiple-type, mixed-strain cultures, even increasing the complexity of the mixture by using from two to four cultures together in their bulk starter tanks?

Basically, a pertinent factor is lack of facilities, equipment, and utensils conceded to be necessary for assuring adequate protection and control of single-strain cultures.

Another factor is the long hours required for completing daily cheese-making operations in many of our factories, plus the necessity of refilling vats during many days of flush production. Clean-up periods and chances to air our factories are cut to a minimum, especially at certain times of the year.

With the general type of equipment, facilities, and practices in this country, mixing of two or more multiple-type cultures has aided many cheesemakers in achieving:

(1) More uniform results in acid development and other desired characteristics of bulk starter prepared daily.

(2) Apparent leveling out of some of the effects of the inhibitory properties of milk. It is, of course, a well-known fact that individual cultures may vary in their sensitivity to inhibitory properties frequently present in milk supplies, and the broader mixture of lactics helps minimize some of the wider swings in starter activity.

From observations made on this practice of mixing two or more cultures, maximum success can be attained when certain conditions are met:

(1) Cultures included within a mixture for starter must be compatible and proven to work well together. It must not be assumed that just any lactic culture will work well with another, because this is definitely not the case.

(2) Cultures used for the mixture should be propagated independently as mother cultures or intermediates and mixed only in the starter tank. If a group of cultures are mixed and propagated as a blend, strain dominance will commonly occur and the mixture of lactics will revert to fewer strains. The benefits of having a wide variety of compatible lactics and associates or citric acid fermenters can be lost rather quickly, sometimes within a propagation or two by cheesemakers.

(3) Protection of all cultures against infection is important. Actual cases of failure have been diagnosed where just one culture out of a good working combination or blend was found to be infected with bacteriophage.

(4) Carrying cultures too long in cheese factories may change the strains of bacteria sufficiently to remove the full benefits of the original mixture. Because of this, some plant operators have adopted the practice of using fresh packages of commercially prepared seed culture each day for making mother culture. This is frequently referred to as intermediate and is made in sufficient quantity for setting up the larger bulk-cheese starter. Mother cultures are not propagated in the usual manner of transferring from flask to flask, but directly from pure seed available in freeze dry form.

A More Specific Form of Mixing Cultures

So far, we have dealt with the mixing of multiple-type, mixed-strain cultures, which is about the broadest type of mixing that we can have. However, foreign literature reports some mixing of compatible single-strain lactics on an alternating basis. It is not unusual for pairs of lactics to be used each day, with enough

[1] Presented at Research Conference held at Beltsville, Maryland, October 30, 1957, by the Eastern Utilization Research and Development Division, USDA, Philadelphia, Pa.

pairs available so that different cultures can be used each day for a week or more without duplication of the lactic strains employed throughout this period.

Currently, some tests are being run on the mixing of selected single-strain lactics, combined with a compatible associate or citric acid fermenting organism. If successful, such a combination will offer a means of much greater control than the broader mixtures of more complex blends. Although it is not exactly the same idea being carried out in foreign countries, it closely approaches the same specific characteristics. In addition, there is the general feeling in this country that the citric acid fermenting bacteria are important for bolstering the hardiness of cheese cultures and for aiding in desired Cheddar cheese flavor. There is reasonably good evidence to justify these feelings on the part of cheesemakers in this country. Development of volatile acids by citric acid fermenting bacteria, combined with lactics, is well known. From the standpoint of activity, we are pleased to present data by F. J. Babel[2] which show clearly the beneficial effects on the activity of a lactic combined with a compatible associate.

Culture, 1% inoculation	Acidity after incubation at 30° C. for: 0 hr.	2 hr.	4 hr.	6 hr.	8 hr.
Streptococcus lactis alone	0.19	0.19	0.23	0.36	0.64
Citric acid fermenter alone	0.19	0.19	0.19	0.19	0.19
Combined culture	0.20	0.21	0.35	0.66	0.82

Standardization of Medium for Cultures and Starters

The problems of assuring a suitable medium are very important.

For sustained good performance of lactic cultures, there is evidence that it is difficult to surpass high-quality skim or whole milk, which has been heated with care and within moderation. However, due to the unpredictable variations which may occur in the growth-supporting properties of natural skim or whole milk, pretested nonfat dry milk solids is being used by many cheese-factory operators. The term pretested must be emphasized, because it obviously needs a more specific definition, or meaning.

In our laboratory, we have experienced wide variations in the qualities of nonfat dry milk solids, presumably pretested for starter activity. It has not been uncommon to observe much wider variations than we desired in the characteristics of cultures propagated in different lots of reconstituted skim. This includes activity, flavor production, and body development.

To give us the confidence we desire, in adopting any given lot of powder for extended propagations of cultures, we want to know that:

(1) The powder selected will support good growth and activity of a *number* of different lactic cultures for at least a week and preferably longer. It is, of course, important to us to prove the merits of any powder for a representative group of cultures, because the reactions of one or two cultures may be quite different from others.

(2) We also inspect all cultures for flavor and body development during the trial test period.

Judging from the results observed, we feel it is in order to suggest a number of important factors which should be determined in pretesting powders for cultures and starters.

(1) Use of a greater number of cultures in testing.

(2) Observing results carefully for a greater number of propagations. A gradual decline in desired qualities of culture may take place.

(3) Testing carefully each new lot of powder against a previously proven lot for activity, flavor production, and body development. This supplies a good base for evaluation.

In England, special culture medium is, or soon will be, offered for preparing cultures and starter for cheesemaking. It is essentially a low-calcium milk solids medium, claimed to be very closely controlled and standardized for reliable culture behavior and protection against phage development.

Protective Equipment, Procedures, and Facilities

Effective control and performance of cheese cultures and starter involve protection against phage infection. We share the opinions of many investigators in referring to bacteriophage as the "Number One" enemy and control problem in the successful development and performance of cultures and starters. Control measures which are being employed in foreign countries and to a growing extent in this country in some degree, include:

(1) Provision of separate facilities for culture and starter preparation, well-isolated from general manufacturing rooms and equipped with independent filtered air inlets or air-conditioning systems.

(2) Rotation of different cultures that have unrelated phage sensitivity patterns.

(3) Use of protective equipment, including:

(a) Water-sealed, air-filtered starter cans with protected inoculating spouts. Their construction is similar to those employed for many years in New Zealand and Australia. They are available from at least one manufacturer in this country.

Briefly, the basic working principles and protective features of this equipment are: The lid, agitator, shaft,

[2] Purdue University, Lafayette, Indiana.

and inoculating spout are all water-sealed to prevent any breathing through these areas. As milk in the starter tank is heated, the expanded air escapes through a small opening at the top provided for this purpose. However, at the end of the heating period, and before cooling is started, a filtering device is attached to the small opening. Therefore, during cooling only filtered air is drawn back into the bulk starter tank. When ready for inoculation, a steam ring is placed around the covered inoculating spout and the water-sealed cover is removed through a jet of steam. The steam is directed upward and across the opening of the exposed inoculating spout to protect the opening against infection from the air. Culture is introduced into the starter tank through the steam-protected inoculating spout and the water-sealed cover is put back into place.

(b) Air-tight equipment which avoids any exchange of air during heating and cooling. Inoculations are made through serum-type rubber stoppers, with the equivalent of hypodermic needles. An integral part of this system is the use of polyethylene bottles, sealed with serum-type rubber stoppers. Specially designed inoculating needles are used for transferring from bottle to bottle as well as bulk tanks. Such equipment is shown in photographs (Figures 1-4) which were taken in a large, modern cheese factory in England. The basic principle involved in the use of this equipment is to avoid any exposure of cultures or bulk starter to air infection. Figure 1 shows 10- and 40-gal. starter cans.

FIG. 1.

Each container is made air-tight by the use of a specially designed tight-fitting closure. A clamping device is used to force a rubber seal against the neck of the cans and a serum-type rubber stopper is used to plug the small opening provided for inoculation.

Figure 2 shows the large tank used for heating and cooling the starter cans of milk, which are completely submerged under water during heating and cooling.

Figure 3 shows polyethylene bottles being used for mother cultures. These range from about 4 oz. for basic seed up to 1-liter (approximately 1 qt.) size for providing the inoculum for bulk cans. The bottles are plugged with serum-type rubber stoppers, and transferring from one to another is accomplished by the use of a specially designed double-needled device, as illustrated.

FIG. 2.

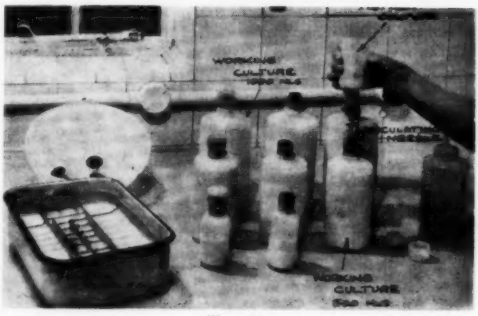

FIG. 3.

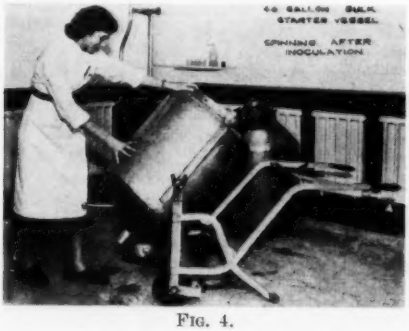

FIG. 4.

The polyethylene bottles are flexible enough to permit discharging the culture when they are squeezed.

Figure 4 shows one of the 40-gal. cans being revolved in a special cradle for mixing the inoculum. The cradle device with wheels also provides a means of transporting the starter cans to a room maintained at 70° F. for incubation.

The figures illustrate the measures which have been taken, principally for protection against phage infection and consequent failures and cheesemaking losses. Whether we agree or not, substantial evidence has been offered for the need of such control measures and the benefits resulting therefrom. Outstanding improvements have been reported following their use in cheesemaking.

If phage-resistant media prove successful for use in this country, phage-control measures may take a different form. However, we need proven results under representative commercial conditions, plus availability of such media.

OUR ASSOCIATION

STUDENT INTERNATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

C. J. Babcock

Director, Poultry Division, Foreign Agricultural Service, USDA, Washington, D. C.

The 23rd Collegiate Student International Contest in Judging Dairy Products, sponsored by the Dairy Industries Supply Association and the American Dairy Science Association, was held in San Francisco, October 21, 1957. The contest was under the supervision of C. J. Babcock, Director of the Dairy and Poultry Division, Foreign Agricultural Service, U. S. Department of Agriculture, assisted by D. R. Strobel, Deputy Director of the Division. The official judges were: *Cheese:* H. L. Wilson, Kraft Foods Company, Chicago, Illinois; *Butter:* N. E. Fabricius, Flambeau Valley Farms, Ladysmith, Wisconsin; *Ice Cream:* J. H. Erb, The Borden Company, Columbus, Ohio, and *Milk:* D. A. Pettee, Creamery Package Manufacturing Company, Boston, Massachusetts. Their associate judges were Neal Angevine, Charles Weinreich, Vern Wilt, and Dan Roahen, from the Dairy Industries Supply Association judging committee.

The efficiency of the judges was checked by Professors E. D. McGlasson and G. H. Watrous for milk; L. J. Manus and W. S. Rosenberger for butter; M. P. Dean and E. W. Custer for cheese, and G. Ilg and W. S. Slatter for ice cream.

The team from the University of Connecticut was first in judging all products and received the all-products bowl and a Fellowship for $1,800 from the Dairy Industries Supply Association. The State College of Washington was in second place and received a Fellowship for $1,650 from the same Association. Cups, wrist watches, and medals for team proficiency in judging single products and for individual accomplishments were scattered widely, as shown by the following listings of the first ten individuals and teams for each of the products and for all products.

CHEESE

Individuals

Rank		*Score*
1.	Robert West, Fresno State College	30.00
2.	Gene Christman, Ohio State University	30.34
3.	Floyd Bodyfelt, Oregon State College	30.55
4.	Ronald Valinoti, University of California	31.01
5.	Edward Geisheeker, University of Connecticut	31.26
6.	Harold Suelter, Kansas State College	31.42
7.	Barney Wallis, Mississippi State College	31.67
8.	Tom Townsend, Kansas State College	31.98
9.	LeRoy Iverson, University of Minnesota	32.51
10.	Lawrence Wills, University of Minnesota	33.26

Teams

Rank		*Score*
1.	Kansas State College	97.71
2.	University of California	98.58
3.	Mississippi State College	102.36
4.	University of Connecticut	102.75
5.	University of Minnesota	104.61
6.	Oregon State College	104.71
7.	State College of Washington	106.35
8.	Ohio State University	107.35
9.	Utah State University	107.94
10.	University of Wyoming	110.33

BUTTER

Individuals

Rank		*Score*
1.	David Duda, Iowa State College	6.00
2.	Larry Weatherly, University of Missouri	7.50
3.	W. Richard DuKelow, University of Minnesota	9.50
4.	Ernest Fleuette, University of Connecticut	10.00
5.	Edward Geisheeker, University of Connecticut	10.51
6.	Robert Ingram, Oregon State College	11.00
7.	Larry Groves, Iowa State College	11.50
T 8.	Larry May, University of Missouri	12.00
T 8.	Gene Christman, Ohio State University	12.00
10.	Keith Yeager, University of Wyoming	12.50

Teams

Rank		*Score*
1.	University of Connecticut	33.50
2.	University of Missouri	33.50
3.	Iowa State College	34.00
4.	University of Minnesota	41.00
5.	University of Wyoming	44.17
6.	Ohio State University	45.17
7.	Oklahoma State University	45.42
8.	Oregon State College	45.50
9.	University of Wisconsin	46.27
10.	State College of Washington	46.34

ICE CREAM

Individuals

Rank		*Score*
1.	Raymond Bay, State College of Washington	29.00
2.	Charles Neikirk, Ohio State University	29.67
3.	Larry Weatherly, University of Missouri	30.00
4.	Arley Olson, State College of Washington	30.84
5.	Richard Fallert, University of Missouri	31.67
6.	Barney Wallis, Mississippi State College	32.00
7.	David Duda, Iowa State College	32.67
T 8.	Patrick McComas, Oklahoma State University	34.17
T 8.	Larry Rayner, Ohio State University	34.17

T 10. Jerry Marcello, The Pennsylvania State University	34.50
T 10. Peter Schoeck, University of Connecticut	34.50

TEAMS

Rank	*Score*
1. Ohio State University	99.01
2. University of Missouri	100.67
3. State College of Washington	100.85
4. University of Connecticut	112.51
5. Kansas State College	114.17
6. The Pennsylvania State University	116.00
7. Mississippi State College	116.01
8. Iowa State College	116.68
9. University of Minnesota	119.76
10. University of Nebraska	122.59

MILK

INDIVIDUALS

Rank	*Score*
1. Tom Townsend, Kansas State College	8.75
2. Maynard Harr, University of Nebraska	9.00
3. Richard Drake, University of Wyoming	9.33
4. Ernest Fleuette, University of Connecticut	9.42
5. Harvey Smith, California Polytechnic College	11.25
6. Patrick McComas, Oklahoma State University	12.50
T 7. Raymond Bay, State College of Washington	12.92
T 7. Donald Raistakka, State College of Washington	12.92
9. Larry Groves, Iowa State College	13.00
10. Charles McGowne, California Polytechnic College	13.25

TEAMS

Rank	*Score*
1. University of Nebraska	37.67
2. State College of Washington	40.09
3. California Polytechnic College	42.33
4. University of California	42.75
5. Kansas State College	44.25
6. University of Wisconsin	46.60
7. University of Connecticut	46.67
8. University of Missouri	49.51
9. Iowa State College	49.84
10. Oklahoma State University	54.25

ALL PRODUCTS

INDIVIDUALS

Rank	*Score*
1. Ernest Fleuette, University of Connecticut	47
2. Tom Townsend, Kansas State College	52
3. Raymond Bay, State College of Washington	57
4. Larry Weatherly, University of Missouri	59
5. Donald Raistakka, State College of Washington	63
6. Gene Christman, Ohio State University	65
7. Lloyd Vierro, University of California	67
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VOL. 41 March, 1958 NUMBER 3

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL HEALTH

107. The relationship of the Whiteside test on bulk herd milk and the incidence of mastitis in the herd. C. JENSEN, I. SCHIPPER, AND P. ASCHBACHER, Dept. of Dairy Husb., N. Dak. Agr. Coll., Fargo. J. Milk and Food Technol., 21, 1: 15. 1958.

The test was conducted by mixing five drops of milk with one drop of *N* sodium hydroxide solution on a glass plate placed on a black background, and the results read after 30 sec. The absence of precipitate is classified as 4+, while a 1+ test is indicative of small particles dispersed throughout the mixture.

The authors believe the test is valuable in detecting mastitis in bulk milk.

H. H. Weiser

BOOK REVIEWS

108. Nutrition Animale. Vol. I. Données Générales sur la Nutrition et L'alimentation. RAYMOND JACQUOT, HENRI LE BARS, AND HENRI SIMONNET. J.-B. Baillière et Fils, Editeurs, Paris 6, France. 472 pp. $13. 1958.

This book is Volume I of a series on animal nutrition, to be published as part of a New Agricultural Encyclopedia. This volume covers in two parts: *(1)* The Nutrients, and *(2)* Digestion and Absorption. The general plan of the series on animal nutrition calls for, in addition to this volume, a continuation Volume II to cover metabolism and requirements and allowances; Volumes III and IV on nutriments and foods, composition, nutritive value, and hygienic qualities of foods; Volume V on nutrition and reproduction; Volume VI on nutrition and lactation (Part 1: milk cow; Part 2: other species); Volume VII on nutrition and growth (Part 1: wool; Part 2: fur animals; Part 3: fish), and Volume IX on nutrition, environment, and health.

This seems to be a very ambitious task, but certainly the resultant books, if critically written, should prove valuable to all workers in the field of animal nutrition. It is regrettable that a series of books such as these are to be published with paper covers.

In the introduction, the authors pose the problem to be considered as that of translating the theoretical nutrient needs of the animals into actual ration formulations. They state that this problem is much more complex than that of nutrition. The first section of the book, after some historical review of classification of nutrients, deals with the nutrients under the headings of: The glucides; The lipids, including a few pages on antioxidants in fats; The protides; The minerals, including water, and The vitamins. Much of the emphasis in this section is on the vitamins, and the authors point out that, although considerable information is available as to the mode of action of at least some of the B vitamins, there is, as yet, no way of relating these functions to the vitamin-deficiency symptoms. Several classification schemes of the vitamins are given. There seems to be no excuse for a classification into "hormovitamins" (those which function in the synthesis or function of hormones) and "enzymovitamins." In the first group are placed vitamins A, D, E, and essential fatty acids. Vitamin C, inositol, citrin, and choline are placed in the latter group. In general, to the reader not working in the field, the impression might be gained from this section of the book that compounds such as choline, inositol, *p*-aminobenzoic acid, citron, adenine, etc., are really vitamins. On the other hand, the historical aspect is interesting to those who have watched many "vitamins" come and go.

Under each of the individual vitamins is discussed very briefly the history, physical properties, stability, units, standards, and natural antivitamins (including many diverse and unrelated topics, such as thiaminase, avidin, etc., although from the headings one might be led to believe that many structural analogs existed in nature). Nothing is included in this section on the function or mechanism of action of any individual vitamins. Presumably, this is to be covered in Volume II, under Part 1 on metabolism.

Following the vitamin chapter is a most interesting, although again not always critical, chapter on feed additives. This includes mention of antibiotics and hormones (surprisingly,

with no mention of stilbestrol or of any estrogen but only of thyroid and antithyroid compounds).

The following chapter deals with methods for the determination of the principal nutrients, mentioning only the types of methods available for dry matter, minerals, total nitrogen, total lipids, crude cellulose, and amino acids and vitamins. No details are given in this brief survey.

Part 2 of the book is devoted to digestion, absorption, and digestive utilization. After a discussion of digestive processes in general, digestion in the ruminant is discussed, with some excellent illustrations of the digestive system of the ruminant, followed by monogastric herbivores, omnivores, carnivores, birds, and the young.

The book then discusses the digestion of the different nutrients, including the glucides, the lipids, the protides, the minerals, and the vitamins.

In general, the book seems to be a useful tool for the nutritionist, and Part 2 appears particularly excellent in its assembly of material in the important field of digestion and absorption of nutrients by the various species.

B. C. Johnson

109. How to Plan and Operate a Soft-Frozen Dairy Products Store. P. H. Tracy and E. T. McGarrahan. The Garrard Press, Champaign, Ill. 1957.

This publication is of interest and benefit to operators, those making plans to become operators of soft-serve dairy product stores, mix manufacturers, and suppliers of equipment. The book also would serve as a useful reference for university personnel teaching ice cream manufacturing, as it is the most complete work of its kind on this subject.

The evolution of the soft-serve store is discussed, and the history of the ice cream industry, from the introduction of the ice cream cone to the new types of freezers, is presented. Many important items such as product freshness, labor-saving devices, and factors favorable to the growth and future development of soft-serve operations are discussed. Since this is a relatively new branch of the dairy industry, the authors have presented detailed information about the cost of setting up a business, as well as operational costs and profit.

A simple but effective accounting procedure for managers and owners is outlined. Such factors as sanitation, employee training programs, advertising, and other considerations involved in planning and operating are presented.

The book is well written, clear, concise, and should be a valuable addition to any dairy library.

L. D. Gibson

BUTTER

110. Maltsmag i smoer (Malty taste in butter). A. Moeller-Madsen, H. Jensen, and A. H. Pedersen, Gov. Research Inst. on Dairy Ind., Hilleroed, Denmark. Res. Bull. 108. 1957. (English Summary)

Prompted by an increase of a malty defect in Danish butter, investigations were carried out to determine the cause of the defect.

Lactic acid bacteria developing a malty flavor in sterile milk were isolated from about 100 of 1,000 milk samples examined. Such bacteria were also always found in malty butter but never in commercial butter starters. The bacteria were identified as strains of *Streptococcus lactis* var. *maltigenes*.

The isolates were more heat-resistant than selected strains of *S. lactis* and *Streptococcus cremoris*. However, all isolates were destroyed by exposure to 63° C. for 30 min. Mixtures of a butter starter and maltigenic strains developed malty flavors after a few transfers when incubated at 30° C., but no malty flavor resulted when the mixtures were incubated at 19 to 22° C., even after 20 transfers.

Chromatographic analysis of steam distillates of malty cultures revealed the presence of 3-methyl-butanal, a compound not found in steam distillates from nondefective starters. These results were in agreement with those of Jackson and Morgan.

Practical experiments indicated that addition of only 5% of malty starter to regular butter starter resulted in butter with malty flavor. Increased sanitation and care during the carrying of the starter and a more frequent change of cultures were recommended to overcome the defect.

T. Kristoffersen

111. Reconstituted butter-like spread. G. H. Norman, J. P. Malkames, and R. Fresnosa, 13th Air Force Central Exchange, Milk Prod. J., **48**, 12: 20. 1957.

The spread is composed of anhydrous butterfat, reconstituted milk, buttermilk (made from reconstituted skimmilk), salt and citric acid. Approximately 50% of the spread is composed of anhydrous fat and about 5% of skimmilk solids. The spread is light yellow in color and the flavor resembles sour-cream butter. The spread is neither mealy nor crumbly, and spreads smoothly.

Details for the manufacture of such a spread are given. The authors feel that this product could be produced on a commercial scale, using an ice cream freezer, acting as the blender, with proper temperature controls.

J. J. Janzen

112. Apparatus for removing butter from the containers of combined churns and butterworkers. P. S. Knudsen. U. S. Patent 2,822,152. 10 claims. Feb. 4, 1958. Offic. Gaz. U. S. Pat. Office, **727**, 1:124. 1958.

Butter is unloaded from the bottom of a combined churn and butter worker by a worm-gear type of butter-propelling device.
R. Whitaker

CHEESE

113. Fibrous structure in cheese curd. N. KING AND J. CZULAK. Nature, **181**, 4602:113. 1958.

The fibrous texture of cheddared curd results from the spreading or flow of the curd granules as they fuse during the cheddaring process. The network of the casein fibrils was studied by microscopic examination by fluorescence methods. The speculation is made that the fibril formation is caused by the orientation of the separate casein molecules and their end-to-end attachment, or by some uncoiling of the polypeptide chains.
R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

114. Process for manufacturing an evaporated food product and the product produced thereby. M. E. ELLERTSON AND S. J. PEARCE (assignors to Carnation Co.). U. S. Patent 2,822,277. 5 claims. Feb. 4, 1958. Offic. Gaz. U. S. Pat. Office, *727*, 1:152. 1958.

Short-time, high-temperature, sterilized evaporated milk is stabilized against storage gelation by heating the milk to from 264 to 285° F. for 70 sec., cooling to about 210° F., then homogenizing, and finally cooling to about 70° F. R. Whitaker

115. Casein-containing lithograph coating. W. H. MARTIN (assignor to Harris-Seybold Co.). U. S. Patent 2,822,280. 10 claims. Feb. 4, 1958. Offic. Gaz. U. S. Pat. Office, **727**, 1:152. 1958.

This patent describes a paper-coating material comprising from 5 to 95% of albumin and from 5 to 95% of casein at pH 8 to 10.3, and sufficient hexavalent chromium compound to provide light-sensitivity. R. Whitaker

DAIRY BACTERIOLOGY

116. Action of rumen fluid on pesticides. In vitro destruction of some organophosphate pesticides by bovine rumen fluid. J. W. COOK, F.D.A., Dept. of Health, Education, and Welfare, Washington, D. C. J. Agr. Food Chem., **5**, 859. 1957.

Parathion and three other compounds containing *p*-nitrophenol and the thiono isomer of Systox were rapidly destroyed, as measured by an anticholinesterase method of analysis and by paper chromatography, when 100 p.p.m. each of parathion and nine other phosphate pesticides were added to aliquots of bovine rumen fluid. The *p*-nitrophenol moiety of the parathion molecule was reduced to *p*-aminophenol. Paper chromatography of extracts of incubation mixtures revealed that thiol isomers are not destroyed like thiono isomers. Evidence indicates that metabolism of parathion in the rumen can account for its apparent lack of toxicity to cattle as reported in the literature. S. Patton

117. Milk irradiation. Irradiation preservation of milk and milk products. J. H. WERTHEIM, R. N. ROYCHOUDHURY, J. HOFF, S. A. GOLDBLITH, AND B. E. PROCTOR, Dept. of Food Technol., M.I.T., Cambridge, Mass. J. Agr. Food Chem., **5**, 944. 1957.

A method was developed which increased the volatility of the off-flavor complex, or its precursors, of irradiated milk so that milk irradiated with 2×10^6 rep of gamma rays was obtained free from detectable off-flavors. The increase in the sensitivity of irradiated milk to browning, caused by the formation of reductones, was further studied. The production of chalky off-flavors from milk fat was caused partly by the formation of peroxides of the more saturated components, whereas oxidized off-flavors were caused by highly unsaturated fractions of the butterfat. A method was found by which milk or milk concentrates might be sterilized by cold sterilization—i.e., by the application of ionizing radiations. S. Patton

118. A growth inhibitor of L. bulgaricus. L. VISWANATHAN AND P. S. SARMA. Nature, **180**, 4598: 1370. 1957.

A peptide-like compound inhibited the growth of this organism. It can be found in skimmilk powder stored 6 mo. at 60° C., in powder heated 5 hr. at 100° C., or when autoclaved 30 min. at 15 p.s.i. pressure. It can be produced by heating lactose with one of several amino acids. Various properties are listed for the material. Its mode of action in retarding growth is being investigated.
R. Whitaker

119. The Bacto-strip technique for making counts of bacteria, molds, and yeasts. C. K. JOHNS AND I. BERZINS, Bacteriol. Div., Can. Dept. of Agr., Ottawa. J. Milk and Food Technol., **20**, 12: 345. 1957.

The Bacto-strip technique can be used with a minimum of laboratory facilities, although considerable manual dexterity is required. The method can be useful in a small dairy plant for routine checking for coliform recontamination of bottled milks. The coliform counts are not comparable to those obtained by the desoxycholate agar plate count method. However, mold counts of air in cheese-curing rooms were comparable to those obtained by standard procedure. The Bacto-strip technique is not a substitute for standard procedures used in the microbial examination of food and other dairy products. H. H. Weiser

DAIRY CHEMISTRY

120. Silage evaluation. Polyunsaturated fatty acids in legume-grass silage. R. M. Ward and R. S. Allen, Iowa Agr. Expt. Sta., Ames. J. Agr. Food Chem. **5**, 765. 1957.

The effects of time after ensiling, presence or absence of preservative, and of crop ensiled on the linoleic, linolenic, and total long-chain fatty acids in legume-grass silage were studied by the use of laboratory silos (glass jars), concrete miniature silos, and a bunker-type silo. There were no major differences between silages with and without added preservatives, as regards the polyunsaturated and total long-chain fatty acids in silages made in the three types of silos. The silage fermentation process caused no major change in the amount of polyunsaturated fatty acid in the total dry matter. However, the percentage of linoleic acid in the total fatty acid of silages was distinctly lower than that in the original forage at the time of ensiling. S. Patton

121. Repellent residues. Determination of small quantities of 2,3,4,5-Bis(Δ^2-butenylene)-tetrahydrofurfural(Repellent R-11) in milk. P. E. Toren and L. D. Goodhue, Research Div., Phillips Petroleum Co., Bartlesville, Okla., and W. R. Kirkham and D. E. Howell, Okla. Agr. Expt. Sta., Stillwater. J. Agr. Food Chem., **5**, 749. 1957.

The insect repellent 2,3,4,5-bis(Δ^2-butenylene)tetrahydrofurfural, (R-11), was tested for its appearance in milk, when used as a spray for dairy cattle. It was administered daily for 5 wk. to dairy cows at a level ten to 20 times that needed for effective fly-control. Analysis of milk samples throughout the treatment period failed to show the presence of any of the repellent wihtin the sensitivity of the method, which was 0.1 p.p.m. The analysis was carried out by separating the butterfat from the milk, reacting an extract of this fat with 2,4-dinitrophenylhydrazine, and measuring the resulting product spectrophotometrically. The validity of determining total R-11 content of milk by analysis of the fat only was demonstrated by measurement of the partition of R-11 between butterfat and aqueous phases. S. Patton

122. First- and second-cycle casein in milk. T. A. J. Payens. Nature, **181**, 4602: 114. 1958.

First-cycle casein (I), made by removing the calcium from calcium-precipitated casein, contains β-casein and α/κ complex. Second-cycle casein (II), made by reprecipitating I with 0.25 *M* calcium chloride at 37° C., does not contain the κ-casein, which remains in solution. The addition of rennin splits the electrophoretic α-peak into two components, α_1- and α_2-casein. It was found that α_2-casein is less abundant in II than in I. α_2-casein very closely resembles κ-casein or is identical to it. The motility of α_1-casein is higher in II than in I. R. Whitaker

DAIRY ENGINEERING

123. Vibrators are production boon. L. S. Metcalfe. Milk Prod. J., **48**, 12:12. 1957.

The author discusses the importance of regulating ingredient flow in the packaging of dehydrated dairy products. The use of vibrators designed for the specific job greatly facilitates the ease and efficiency of modern-day automatic handling and packaging. Some of the problems involved, such as noise, air requirements, and design, are discussed. J. J. Janzen

124. Frozen-confection mold filler. M. Hughes. U. S. Patent 2,820,490. 3 claims. Jan. 21, 1958. Offic. Gaz. U. S. Pat. Office, **726**, 3:515. 1958.

The frozen-confection mold filler consists of a tray having the same breadth and width as the mold, having round holes in the bottom directly over the molds, cylindrical chambers extending downward from the holes, ball-type valves closing the bottom of each cylinder, and a ball-lifting device which is actuated by placing the tray on the mold. The size of the chambers determines the charge of mix delivered into each mold. R. Whitaker

125. Carton-handling apparatus. H. E. Knappe (assignor to Cherry-Burrell Corp.). U. S. Patent 2,821,824. 9 claims. Feb. 4, 1958. Offic. Gaz. U. S. Pat. Office, **727**, 1:37. 1958.

A device is decribed for moving a Cancotype paper milk bottle along a conveyor line, opening the lid for filling, closing the lid after filling, and providing a no-fill feature if the lid is improperly opened at time of filling. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

126. An economic study of small fluid-milk-plant problems in northern Idaho. W. J. Monroe and S. A. Walker. Idaho Agr. Expt. Sta., Bull. 255. 1956.

A study was made of the costs of procuring and processing milk in six plants in an isolated area. Analysis of the data showed that large plants have a marked economic advantage over small plants. In making the analyses, model plants were synthesized and it was found that total production costs ranged from $71.45 per 1,000 lb. of milk for a plant supposedly processing 9,080 lb. of milk weekly, and $29.16 per 1,000 lb. for a plant planned to process 145,280 lb. per week. When all costs were considered, it is stated that any plant processing less than 41,000 lb. per week can not cover all of its costs. A. W. Rudnick, Jr.

127. Procurement policies and practices of a selected group of dairy processing firms. Part I. Some aspects of market structure, competitive behavior, and market results. R. L. Clodius, D. E. Fienup, and R. L. Kristjonson. Wis. Agr. Expt. Sta., Research Bull. 193. 1953.

In a comprehensive, but preliminary, survey of one section of Wisconsin, the authors have attempted to define the milk-marketing process. In a realistic manner, they have taken into consideration the many factors other than price that affect the market. Further study is needed to determine the exact means which management uses to establish price and which producers use in determining markets.

It was demonstrated that many factors are at work which force dairy plants to increase in size. The authors advise further study in this area. They also consider that certain marketing problems will be solved by circumstances. The authors indicate that attitudes which assume that time alone, if long enough, will solve all problems are not the approach likely to satisfy either the farmers or the firms in the field, or the social scientists. The challenge is to see what can be done quickly.

A. W. Rudnick, Jr.

128. Vermont's dairy industry. R. O. Sinclair. Vt. Agr. Exp. Sta. Bull. 588. 1956.

This is a history and economic survey of the dairy industry in Vermont. The author predicts a decrease in number but increase in size of both dairy farms and milk handlers.

A. W. Rudnick, Jr.

129. Cooperative and nonprofit dairy associations in Michigan. O. Ulrey, and A. Howland. Mich. Agr. Expt. Sta. Spec. Bull. 404. 1956.

After making a broad survey of types of dairy cooperatives in Michigan, the authors recommend that dairy farmers make greater use of the cooperative enterprises. They also recommend an increase in the size of cooperatives, to obtain greater efficiency and lower costs.

Further studies are advised, to determine number and location of multi-function dairy cooperatives that will be in a position to engage in the competitive fields of milk processing and distribution. A. W. Rudnick, Jr.

130. An analysis of milk-vendor operations in South Carolina. J. H. Padgett. S. C. Agr. Expt. Sta. Circ. 100. 1955.

This survey of vending-machine operations in South Carolina gives consideration to factors making this type of business profitable. The data indicate that a milk dealer should have a minimum of 20 machines in order to furnish sufficient business for one full-time man. Location should be chosen with consideration of potential volume of business, distance from point of supply, and protection against pilferage.

The author considers that this type of operation is one that can be profitable and one which can open new markets for Class I milk in the state. A. W. Rudnick, Jr.

131. Container and blank with pouring means. F. E. Makuta (assignor to Ex-Cell-O Corp.). U. S. Patent 2,820,581. 2 claims. Jan. 21, 1958. Offic. Gaz. U. S. Patent Office, **726**, 3: 539. 1958.

An opening in the side of the gable of a Pure-pak type paper milk container for pouring purposes is described. The opening is closed by a tab of paper board designed to protect the pouring lip until the tab is lifted.

R. Whitaker

FEEDS AND FEEDING

132. Dietary carbohydrates. A review of the effects of different carbohydrates on vitamin and amino acid requirements. A. E. Harper and C. A. Elvehjem, Dept. of Biochem., Univ. of Wis., Madison. J. Agr. Food Chem., **5**, 754. 1957.

Literature on the effects of different carbohydrates on vitamin and amino acid requirements has been reviewed. A substantial body of evidence indicates that when a less soluble carbohydrate is substituted in the diet for a more soluble one, the requirements for most members of the vitamin B complex and for essential amino acids, reported as a percentage of the diet, decreased. The effect of complex carbohydrates in lowering vitamin B requirements has been related to changes in the intestinal microflora; however, there is no evidence to suggest that such changes influence amino acid requirements. Instead, the lower amino acid requirements appear to be a result of physiological effects on food intake, digestion, or absorption. Thus, although dietary carbohydrate serves primarily as a source of calories, indirect effects of individual carbohydrates may be of considerable nutritional significance. S. Patton

133. Quality of plant proteins. Protein quality of oat varieties. E. B. Weber, J. P. Thomas, R. Reder, A. M. Schlehuber, and D. A. Benton, Dept. of Agr. Chem. and Agron., Okla. A and M Coll., Stillwater. J. Agr. Food Chem., **5**, 926. 1957.

Comparison of 16 varieties of oats was made by feeding them to groups of rats, as their sole source of protein. Consistent differences were observed between the nutritive values of some of the varieties in four experiments. The levels of lysine and methionine in the protein of the oat varieties were determined, but there was no correlation between the weight gains of the rats fed these oat samples and the levels of lysine and methionine in the grain. Time and

location of planting oats were not important factors in determining the nutritive value of the oat protein. S. Patton

134. Effect of age of young dairy calves on dry-matter digestibility of alfalfa pellets. M. E. McCULLOUGH AND L. R. SISK. Nature, **181**, 4601:57. 1958.

Calves were offered whole milk and alfalfa-meal pellets free-choice from birth to 12 wk. old. The pellets were unpalatable until the calves were 10-12 wk. old. There was little or no change in dry-matter digestibility from 6 to 12 wk. R. Whitaker

135. Effects of fiber content of the ration on milk production and hot-weather discomfort of producting dairy cows. R. E. LEIGHTON AND I. W. RUPEL. Texas Agr. Expt. Sta., Prog. Rept. 1889. 1956.

Cows were fed diets equal in protein and total energy but differing markedly in fiber. In hot weather, the cows on low fiber produced more milk (2.16 lb. per day) and had lower rectal temperatures, respiration rates, and pulse rates. There were no detectable differences in production during mild weather.

A. W. Rudnick, Jr.

136. Experimental production of abnormal, premature calves and retained placentas by feeding a species of perennial broom. J. W. DOLLAHITE AND W. V. ANTHONY. Texas Agr. Expt. Sta. Prog. Rept. 1884. 1956.

By feeding broomweed (*Gutierrizia microcephala*) the authors caused cows to produce a high percentage of premature, weak, or dead calves. Abortions in early stages of pregnancy also were produced experimentally.

A. W. Rudnick, Jr.

137. Effects of Marcol B-75 in a whole milk replacer on growth and scours in dairy calves. R. E. LEIGHTON. Texas Agr. Expt. Sta. Prog. Rpt. 1877. 1956.

Dairy calves suffering from scours resulting from overfeeding of whole-milk replacers prepared from dried skimmilk and dried whey recovered within 48 hr. when Marcol B-75 (fat source) was included in the diet.

A. W. Rudnick, Jr.

GENETICS AND BREEDING

138. The effects of breeding condition of cow at time of artificial service and treatment of semen on nonreturns and nonreturn decline. F. H. FLERCHINGER, R. E. ERB, AND M. H. EHLERS. Wash. Agr. Expt. Sta. Tech. Bull. 19. 1956.

Observations were made on 11,956 first- to fourth-service cows. First service for 2,488 normal cows averaged 70.1% 180-day nonreturns; whereas, 5,061 cows classified as abnormal averaged 64.1%. The nonreturns declined 10.7% between 29 and 180 days for normal cows and 13.7% for abnormal cows. When there were multiple abnormalities, the per cent nonreturns ranged as low as 51% and the decline after 29 days was in excess of 20%. The fertility of heifers was 0.3% lower than that of cows. The inseminating tube could not be passed through the cervix in 40% of the heifers and in 4% of the cows. Fertility was 8% lower for these animals. Bulls of high fertility showed a lower decline in nonreturns between 29 and 180 days. Antibiotics in the semen increased fertility and reduced the decline after 29 days, especially when used on abnormal cows. A. W. Rudnick, Jr.

HERD MANAGEMENT

139. Dairy cattle selection, type classification, and production records. H. W. HALL AND H. C. DICKEY. Maine Agr. Expt. Sta. Bull. 546. 1956.

After analyzing pedigrees of 531 Holstein sires and those of their mates, formulas were evolved to be used in predicting transmitting ability. These were *(1)* Averaging the records of the daughters of the sire's ancestors for three generations; *(2)* summation of 50% of A's equal-parent index, 25% of B's equal-parent index, 10% of D's record, 5% of E's record, and 10% of C's equal-parent index; *(3)* Method 2, with the use of regression indexes instead of equal-parent indexes; *(4)* average of A's regression index and Dam D's record, and *(5)* average of Sire A's regression index and Dam D's regressed record. (A is bull's sire; B, maternal grandsire; D, dam; E, maternal great-granddam, and C, maternal great-grandsire.)

Methods 1 and 3 correlated highly with the actual record. Where complete information is lacking in the pedigrees, Method 5 can be used, if there is a proving on the sire and a record of production on the dam of the bull.

A. W. Rudnick, Jr.

140. Growth measurements of Holstein females from birth to seven years. H. P. DAVIS AND I. L. HATHAWAY. Neb. Agr. Expt. Sta. Research Bull. 177. 1955.

This is a comprehensive study of growth of 262 cows from birth to 84 mo. or disposal. Patterns of growth based on monthly measurements are discussed. A. W. Rudnick, Jr.

141. Milk-strainer cover. J. J. REGAN. U. S. Patent 2,822,107. 3 claims. Feb. 4, 1958. Offic. Gaz. U. S. Pat. Office, **727**, 1:111. 1958.

To protect milk from the atmosphere, a cover, and a means of removing it mechanically, is provided for the top of a milk strainer designed for use on milk cans.

R. Whitaker

ICE CREAM

142. The call-order system. G. P. CHIPMAN, The Denominator Co., Inc., New York, N. Y. Ice Cream Rev., **41**, 7: 43. 1958.

The call-order system for the distribution of ice cream offers better service to the customer, because he will receive exactly what he has ordered; substantial savings for the distributor, through reduction in the number of routes or in the number of trucks required; more frequent contacts with each customer, because he is contacted regularly via the telephone; possibility of elimination of inventories of special flavors more readily; increase of the area serviced by a delivery truck, as it is unnecessary to call on accounts which do not have an order on a given day; facilitation of product planning, in that the manufacturer can achieve wider distribution of a new flavor or specialties; easier production planning, because sales requirements are known a day in advance; virtual elimination of returns, and removal of the possibility of favoritism from route operation.

The procedure for setting up a successful call-order system involves careful planning at least one year in advance of the time the system goes into effect. Each customer must be sold on the method. Under this system the customer becomes his own merchandising manager. Such details as billing forms to be used and the time when accounts will be contacted must be planned in advance, as well as the system to be employed in translating phone-order forms into a truck-loading sheet.

W. J. Caulfield

143. What is the value of vacuum treatment on ice cream mix? H. E. BEHLMER, Cherry-Burrell Corp. Ice Cream Rev., **41**, 7: 52. 1958.

The chief advantage cited for the use of a vacuum treatment in processing ice cream mix is improved flavor. Many of the feed and weed flavors are volatile and can be removed by a suitable vacuum treatment. Heat in the form of setam is mixed directly with the product, to raise the temperature to the desired level in the processes described. Live steam is considered ideal for this purpose, because it provides for instantaneous heat transfer, and possible danger of heat damage thus is minimized. Expansion and increased velocity of the product and steam under a partial vacuum results in division of the milk product into exceedingly fine particles, thus greatly increasing the surface area of the milk product, which speeds up evaporation. The use of steam also permits application of the principle of steam distillation, in the removal of flavor substances having low vapor pressure. By passing heated steam through heated liquid, the steam carries away materials by forming volatile mixtures having a lower boiling point than the material itself. Thus, the use of steam plus vacuum makes possible the removal of off-flavors from milk and its products which would not be possible if vacuum alone were employed.

The details of the operating principles involved with the vacreator and the Aro-Vac equipment are discussed. W. J. Caulfield

144. Liquid and bulk sugar handling in the ice cream plant. W. H. OTTEY, Spreckles Sugar Co., San Francisco, Calif. Ice Cream Rev., **41**, 7:87. 1958.

Savings in the initial purchase price of the sugar, and savings in labor and in space in the plant, can be effected through the use either of liquid sugar or of a bulk sugar installation. The cost of a liquid sugar installation, including a 5,000-gal. storage tank, pump, fittings, and a meter is estimated at $3,500–$4,000. The cost of a bulk sugar installation of the simplest design would be approximately $700–$10,000. Plants using 500,000 lb. of sugar per year can profitably use a liquid sugar installation; whereas, a bulk sugar installation can be used in plants using 800,000 lb. or more of sugar per year.

W. J. Caulfield

145. Centralization of processing and distribution. V. F. HOVEY, JR., General Ice Cream Co., Schenectady, N. Y. Ice Cream Rev., **41**, 7:42. 1958.

To achieve greater efficiency in its operations. the General Ice Cream Company has consolidated the operations of 28 plants into nine plants. This consolidation has resulted in a 25% reduction in personnel, a reduction in inventories, a smaller staff, and has made it economically sound to install larger and more efficient processing and office equipment.

The changes in organization and method of operation, since the consolidation, make use of a production coordinator for each manufacturing plant, to maintain production and transport schedules, and to maintain proper inventories at the branch distribution points; mobile pallets, which have been introduced for handling the finished product and which make it possible for two men to load and unload a 4,500-gal. transport in 20 min.; an increase in a complete turnover of stock in the hardening room, from three times to six times per month; the closing of branch offices, and the settling of drivers' accounts and the billing of customers from central offices; the closing of many of the distribution branches. (Route days have been reduced 25%, and the gallonage per route has been increased by 33%); the farming out of much of the repair work on trucks now, with the net result of a reduction in costs; the reduction of office personnel by 25%, and the reduction of the product line once (and it is in the process of being drastically reduced a second time at present).

It is the author's belief that the industry can no longer afford the luxury of small plants, small offices, and a large supervisory staff. The product line, as well as some of the services, must be curtailed if the manufacturer is to supply ice cream at supermarket prices.

W. J. Caulfield

146. Stabilizer for frozen, sweet aqueous-base comestibles, and product and method utilizing the same. W. S. Steinitz (assignor to American Food Labs., Inc.). U. S. Patent 2,823,129. 16 claims. Feb. 11, 1958. Offic. Gaz. U. S. Pat. Office, **727**, 2:379. 1958.

This patent covers a liquid ice cream stabilizer composed of gum or other material used as a stibilizer for ice cream, suspended in a water-free organic liquid. R. Whitaker

147. Stabilized and bodied ice cream. H. A. Toulmin, Jr. (assignor to The Commonwealth Engineering Co.) U. S. Patent 2,823,128. 8 claims. Feb. 11, 1958. Offic. Gaz. U. S. Pat. Office, **727**, 2:379. 1958.

Water-soluble NRRL dextran of high molecular weight is used as an ice cream stabilizer at the rate of 0.01 to 0.5%. R. Whitaker

148. Process of making frozen desserts and the product thereof. L. D. Hilker (assignor to National Dairy Products Corp.). U. S. Patent 2,821,480. 25 claims. Jan. 28, 1958. Offic. Gaz. U. S. Pat. Office, **726**, 4:771. 1958.

Ice cream mix is improved through the use of a blend of an edible aliphatic fatty acid monoester, containing not more than 10% nonmonoester material and a full aliphatic fatty acid ester. The addition of at least 15% of the full ester to the monoester aids in dispersing the difficultly dispersible monoester.

R. Whitaker

NUTRITIVE VALUE OF DAIRY PRODUCTS

149. Emergency feeding in disaster. D. L. Bovee, Food and Nutrition Consultant, American Red Cross. Am. J. Clin. Nutrition, **6**, 1:77. 1958.

In Red Cross experience, it has been amply demonstrated that following disaster, immediate provision of food and drink helps to relieve tension, allay feeling of anxiety, and boost morale. And there are special food needs for infants and small children, for pregnant and nursing mothers, and for others, who may be ill or injured. Milk is important for use, as such, for preparation of infant formulas, and in the preparation of other foods and beverages. Evaporated milk should be made available in proportion of one tall can per day for each child under 2 yr. of age. A nursing mother should be given, in addition to her allowance, the allowance of one child. The dry milks are important also, when stored in sealed containers. Such containers protect the contents from radioactive contamination. In case the package has been exposed to such contamination, the exterior should be washed with detergent solution, to remove any radioactive substance before opening.

For individuals in need of high-protein feedings, the author recommends either a mixture consisting of one tall can of evaporated milk, 3¼ cups of dry skimmilk plus 14½ oz. of water, or three cups of dry skim, mixed with three cups of whole milk. Either mixture will provide 150 g. protein per quart.

Frank E. Rice

150. Milk allergy—diagnostic aspects and the role of milk substitutes. J. H. Fries, New Hyde Park, N. Y. J. Am. Med. Assoc., **165**, 12:1542. 1957.

In the author's own medical practice, as an allergy and pediatric specialist, less than 0.3% of his patients have been allergic to milk. He deplores the growing tendency to assume an allergy to milk in infant feeding, based on symptoms that might have other explanations. He estimates that about 5% of the difficult feeding problems in his practice might be diagnosed by some physicians as due to cow's-milk in the formulas, whereupon milk would be removed and vegetable products such as soy beans, or meat, would be substituted. Gastrointestinal disturbances observed in such cases may be owing to poor formula-preparation, excessive feeding, bacterial contamination, physical properties, special toxins, etc. To diagnose allergy as a result of the cessation of symptoms with the withdrawal of milk is fallacious.

Sensitivity to milk is generally owing to lactalbumin. In such cases, evaporated milk or boiled milk is acceptable, because the heat to which the milk has been exposed renders it relatively nonatigenic. Other mammalian milks may be acceptable, since lactalbumin is a species-specific protein. Conversely, the caseins of all mammals are alike antigenically. Sensitivity to this component implies sensitivity to all mammalian milks. Furthermore, casein is not rendered nonantigenic by the application of heat. Fortunately, however, casein-sensitivity is rare; its occurrence is even doubted by some authors.

It is not safe to withdraw milk from the diet of the young for a long period of time. Where it is withdrawn, it should be retried at frequent intervals, because infants frequently lose their sensitivity. It is only when attempts to use mammalian derivatives fail, that the use of a vegetable-base milk should be considered.

The nutritive quality of the protein in soy beans is high but not as high as that of milk. The protein of the soy bean loses in nutritive value following its exposure to the high heat

employed in processing. Short-term studies on animals and observations on babies indicate that the protein of the soy bean may be nutritionally satisfactory. Long-range observations are yet to be made on children grown to adolescence, who have been deprived of mammalian milk for long periods during infancy.

Sensitivity to soy bean products may occur in infant feeding, though not frequently. When they do occur, as they may on prolonged use, cross-sensitivity to other legumes also may be found. The soy bean can provoke gastrointestinal disturbances, owing to cellulose or to certain deleterious substances in the bean which may not be entirely destroyed by the degree of heat to which the bean is exposed in processing. Frank E. Rice

SANITATION AND CLEANSING

151. The influence of water temperature and hardness on the cleaning of C-I-P farm milk pipelines. H. E. CALBERT, Dept. of Food Ind., Univ. of Wis. J. Milk and Food Technol., **21**, 1:12. 1958.

A laboratory model of a C-I-P milk pipeline outfit was used in studying the sanitary efficiency on the farm. It was demonstrated that the selection of proper cleansing agents, the periodic use of acid cleanser, the control of temperature in the range of from 130 to 140° F., and cooling to 90° F. during the washing cycle yielded satisfactory results.

H. H. Weiser

152. Survey of plant cleaning. J. G. SCHAHFER, Seco Chemicals Inc., Detroit. Milk Prod. J., **48**, 12:16. 1957.

The author reviews the present situation pertaining to the use of chemical sanitizers in the dairy industry. The use of hypochlorites is discussed at length. Brief consideration is given to other halogens and derivatives, such as iodine and the chloramines. The use of quaternaries also is discussed.

Industry's microbiological problems are discussed under the following headings: Coliforms, thermoduries and thermophilies, psychrophiles, bacteriophage, and yeast and molds. J. J. Janzen

153. Can washer. M. A. BECKER (assignor to Beam Chemical Co.). U. S. Patent 2,821,988. 6 claims. Feb. 4, 1958. Offic. Gaz. U. S. Pat. Office, **727**, 1:80. 1958.

The interior of milk cans in an inverted position is sprayed with a detergent solution by means of a rotating spray-nozzle.

R. Whitaker

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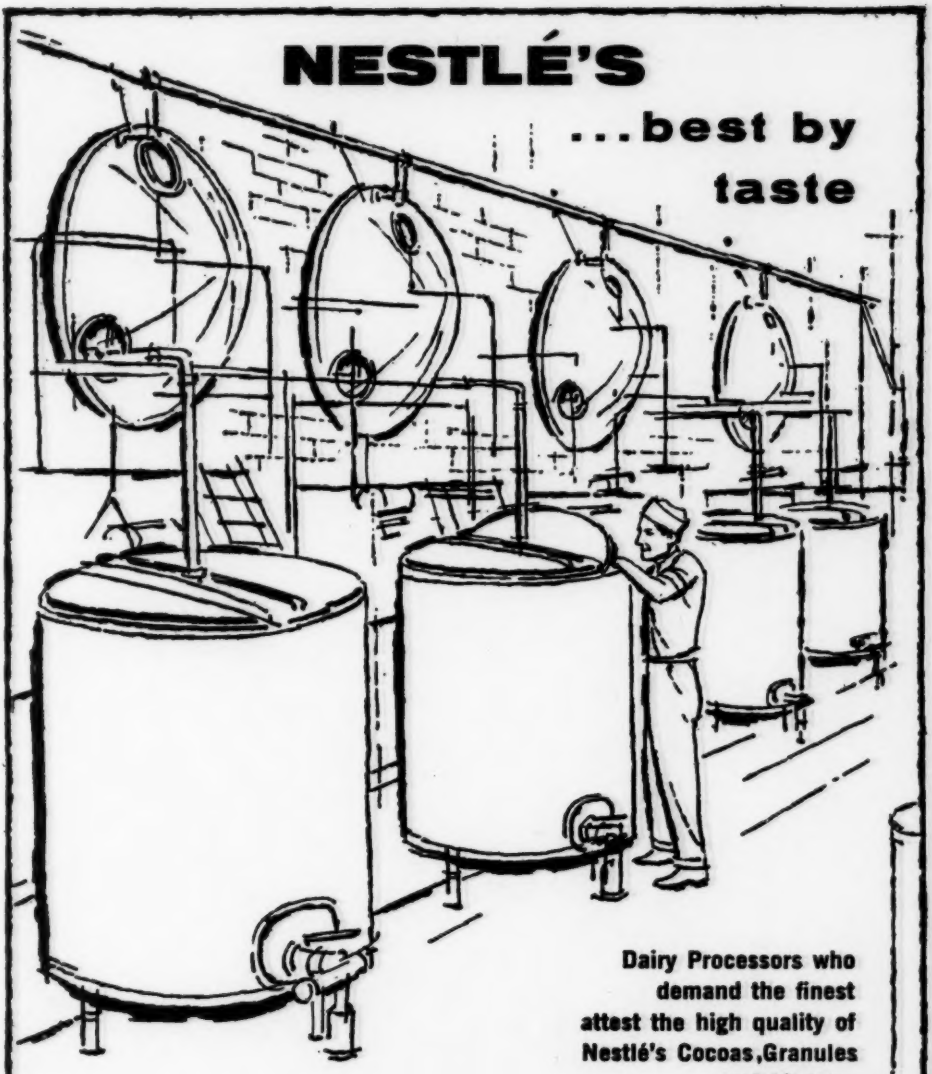

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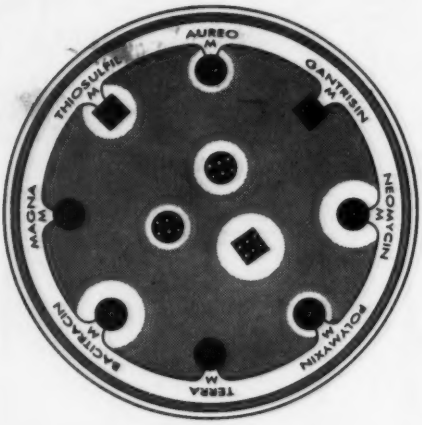

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